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CERTAIN EFFECTS OF ULTRA-VIOLET IRRADIATION ON THE CHEMICAL AND NUTRITIVE PROPERTIES OF BAKED PRODUCTS[1]

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Introduction

The independent and almost simultaneous announcement by Hess and Steenbock that antirachitic potency could be conferred on food products by exposure to ultra-violet light stimulated marked interest in this special field of investigation. Realizing the commercial possibilities and the possible benefits to public health, Steenbock filed application on June 30, 1924, for patent rights under the title "Antirachitic Product and Process." The patent as granted (M. S. Patent 1,680,818) relates particularly to a method of preparing antirachitic products of an edible character such as foods and medicines. The experimental results to be reported in this paper are primarily concerned with the activation of the "Premium" type of soda cracker. In the main, the studies have dealt with the time period required to induce an approved degree of potency in accordance with the standard technique established by Steenbock, and the effects of irradiation on the keeping qualities of the baked goods and of the two types of shortening generally used in their manufacture.

The process has to do with the production of vitamin D from small amounts of ergosterol, and possibly its derivatives, in the food materials, with a view to supplying the human dietary with an attractive and palatable source of this essential factor which has a limited distribution in our naturally occurring foods. A great mass of literature has accumulated since the identification of this vitamin factor as a distinct entity

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by McCollum in 1922 and it is not possible to attempt a complete review of it here.[3] Clinics and laboratories of pure as well as of applied science have contributed extensively to the publications in this field. The investigation of rickets is an outstanding example of the value of interweaving the basic sciences with clinical medicine.

The scientific literature which has more or less of a bearing on this paper has developed through several stages dating from the identification of vitamin D in 1922 to the present time. The chief lines of development have centered on (1) experimental rickets, (2) phosphorus and calcium metabolism in rickets and their variations in the blood, (3) interrelation of vitamin D and light, (4) chemical nature of vitamin D, (5) irradiated foods and irradiated ergosterol, and, (6) methods of assaying the antirachitic factor.

Mellanby (1919), working with puppies, showed that cod liver oil and butter fat possessed antirachitic value. It was his opinion that rickets was caused by insufficient intake of an antirachitic factor which he believed was probably vitamin A. For a time this view was generally accepted. Hess and Unger (1919, 1920) pointed out that infants sometimes developed rickets despite the fact that they had received enough milk to provide what would seem to be a sufficient amount of vitamin A.

Sherman and Pappenheimer (1921, 1921a) reported that rickets in rats could be caused or prevented by altering the mineral components of a ration practically free from fat-soluble vitamins, but it has been recognized that their animals still retained some body storage of these vitamins. A little later in the year Shipley, Park, McCollum, and Simmonds (1921) reported that the phosphate-ion may be the etiological factor in certain cases of rickets. Later still, McCollum, Simmonds, Shipley, and Park (1921, 1922) found that 1% cod liver oil was equivalent to from 10% to 20% of butter fat in dealing with a very low calcium supply, and pointed out that cod liver oil is rich in some factor which butter contains in only slight amounts, but which exerts a marked influence on the bone development of rats subsisting on low calcium diets. They regarded this calcium-depositing or phosphorus-mobilizing factor as probably distinct from fat-soluble vitamin A. Hart, Steenbock, and Hoppert (1921), in connection with their calcium balance experiments with milk-goats, reported a calcium-conserving factor in

[3] More complete bibliographies and discussions of the various related phases of vitamin D investigations are to be found in such familiar publications as the Treatise on Rickets, Osteomalacia and Tetany, by Hess (1929); the monograph by Parks on The Etiology of Rickets, published in Physiological Reviews 3, 1923; The Vitamins, by Sherman and Smith (2d ed., 1931); Ultra-Violet Light and Vitamin D in Nutrition, by Blunt and Cowan; The Newer Knowledge of Nutrition, by McCollum and Simmonds (4th ed., 1929); The Vitamins, by Ethel Browning (1931). Convenient citations to the literature appear in Wodlinger's Bibliographical Survey of Vitamins 1650–1930 (1932).

cod liver oil, green oats, and carefully cured oat-hay, which they regarded as distinct from vitamins A, B, and C.

McCollum and his co-workers (1922) established vitamin D as a separate entity by destroying vitamin A in cod liver oil through mild oxidation, without affecting the potency of the bone growth factor in the oil. In conjunction with their study of the relative anti-xerophthalmic and growth promoting values of different oils they also employed the "line test" for measuring the calcium depositing power of these same oils in the bones of rachitic animals on diets very deficient in calcium.

The extensive experiments of Steenbock and Nelson (1923) on the relation of light to ophthalmia and growth confirmed McCollum's position that the antirachitic factor is distinct from vitamin A.

Interrelation of Vitamin D and Ultra-Violet Light

Huldschinsky (1919–20) showed by means of radiographs that ultra-violet light caused complete healing of rickets in children after eight weeks. Hess and Unger (1921) confirmed the work of Huldschinsky by curing rickets in infants by exposing them to sunshine or ultra-violet light without any alterations in their rickets-producing diet. Howland and Kramer (1921) reported a decrease of the inorganic phosphate in the blood serum of infants suffering from rickets, but during healing the content of phosphorus gradually increased to the normal level. Hess and Gutman (1922) found that the healing of rickets by sunlight induced the inorganic phosphate of the blood to increase to its normal level. A little later in the year Hess and Lundagen (1922) reported a seasonal tide of blood phosphate in infants correlated with the seasonal variation of ultra-violet waves in the sun's spectrum.

From 1921 to 1924, inclusive, ample evidence was produced by numerous investigators to show that irradiation of the body with short wave lengths from sunlight or from artificial lamps stimulated growth and bone development in children, rats, chicks, and pigs regardless of their subsistence on diets containing unsatisfactory relations of calcium and phosphorus. Blood and bone analyses and x-rays were the tools employed in measuring the effects.

Irradiation and Vitamin D

The first public announcement relative to conferring antirachitic potency on food materials by irradiating them with ultra-violet light was made by Hess (1924) in an address read on June 7. Steenbock's original announcement appeared September 5, 1924. Hess and Weinstock published a complete account of their studies in December, 1924. They successfully activated several oils, lettuce, and growing wheat

plants. Rats on a drastically rachitic diet were protected against rickets by the daily consumption of 0.1 cc. of irradiated cottonseed oil. The activation of the unsaponifiable portion of vegetable oils led them to investigate next (1924) the question of irradiating cholesterol and phytosterol, the main constituents, respectively, of the unsaponifiable fraction of animal and vegetable fats. They reported that 0.25 cc. of an irradiated 1% suspension of highly purified phytosterol and cholesterol contained some ergosterol. Rosenheim and Webster (1925), also Steenbock and Daniels (1925), reported similar results. In their study of the induction of calcifying properties in a rickets-producing ration by radiant energy Steenbock and Nelson (1924) found that a rachitic ration could be made antirachitic by ultra-violet irradiation.

Chemistry of Vitamin D and Irradiated Ergosterol

McCollum and his co-workers (1922) demonstrated the stability of vitamin D to oxidation; Zucker, Pappenheimer, and Barnett (1922) established its stability to saponification; Dubin and Funk (1923) to hydrogenation; and Nelson and Steenbock (1925) found that the vitamin D factor in cod liver oil was not precipitated by digitonin. Hess and Weinstock (1925) found that the radiations which activated cholesterol were essentially the same as those which protected animals against rickets when they were exposed to the direct rays. By means of spectral absorption tests they found that activated cholesterol absorbed ultra-violet radiations to a less degree than did ordinary cholesterol. Activation was caused by rays between 200 and 290 millimicrons, but between 254 and 265 millimicrons the rays were utilized to the extent of 90% in the process.

Steenbock and Black (1925) showed that cod liver oil lost its antirachitic potency by excessive irradiation.

Schlutz and Morse (1925) called attention to the possibilities that a small amount of some particularly absorptive impurity which followed through repeated crystallizations of cholesterol from alcohol might be the factor responsible for the antirachitic potency induced by irradiation and the changes caused in the absorption spectrum.

By precipitation with digitonin Hess, Weinstock, and Sherman (1926) removed inactive cholesterol from their irradiated "cholesterol." The active non-precipitable fraction was only 4% or 5% of the original cholesterol. Rosenheim and Webster (1927), using the same means of separation, reported that only about 0.1% of their original cholesterol remained in the active fraction. They considered the double bond and the OH group to be essential in the provitamin.

Hess and Windaus (1926) reported that chloesterol or phytosterol could not be activated, following two precipitations as the dibromide.

In two subsequent papers (1927a, 1927b) they stated that ergosterol with three double bonds and one hydroxyl group could be activated, and that cholesterol, as usually prepared, contains some ergosterol as an impurity. Hess (1927) and Windaus (1927) reported simultaneously that irradiation of ergosterol altered its absorption spectra; Heilbron, Kamm, and Morton (1927, 1927a, 1927b) reported similar results, and Rosenheim and Webster (1927) showed that pure cholesterol does not exhibit the characteristic absorption bands heretofore assigned to it, and the purified product cannot be activated.

Rosenheim and Webster (1928) expressed their belief that the typical ring structure of ergosterol and the relative positions of its three double bonds were factors responsible for the conversion of a sterol into vitamin D.

Windaus and Linsert (1928) showed that irradiated ergosterol increased in solubility, its absorption spectra changed, its molecular weight and number of double bonds remained the same, but these latter might shift inside the molecule as might also the alcohol group since digitonin no longer precipitated irradiated ergosterol.

Rosenheim and Adam (1929) concluded from their study of monomolecular films of irradiated ergosterol in relation to the production of vitamin D, that it was not possible to decide which of the three constituents of the active mixture, as pointed out by Bourdillon, Fischmann, Jenkins and Webster (1929), may represent the vitamin. The vitamin may or may not be a highly absorptive unsaturated ketone or a by-product of its formation, but, whatever the case, it is believed that the lability of the hydroxyl hydrogen in the CH(OH) group plays the controlling role in the molecular changes caused by irradiation.

In two papers dealing with the distillation of vitamin D and the effects of a first long wave irradiation of ergosterol followed by a second irradiation of the resulting products by short waves, Askew, Bourdillon, Bruce, Jenkins, and Webster (1930, 1930a) presented a method for separating the irradiation products of ergosterol "by distillation and fractional condensation in a high vacuum." They prepared a very potent crystalline product melting at 113° to 115° C. They considered it possible that several antirachitic substances may have been formed from ergosterol, and that their crystals may have been merely a fairly pure specimen of one of the formation products. In their second paper they reported that vitamin D is not the substance with an intense absorption at 280, which is produced by short wave irradiation on some product induced by the preceding action of long waves.

Experimental evidence indicating that vitamin D is a simple isomer of erogsterol, and that it may exist in more than one form was reported by Bills, McDonald, DeMiller, Steele, and Nussmeier (1931).

Askew, Bruce, Callow, Philpot, and Webster (1931) prepared a very potent crystalline form of vitamin D which they named "calciferol." This was found to be more potent against rickets than the crystalline D_1 form of Windaus, Lüttringhaus, and Deppe (1931), whose preparation had a higher optical rotation, but a lower antirachitic activity and absorption coefficient. Calciferol, however, did closely resemble the crystalline D_2 preparation of Linsert which Windaus and associates discussed in an addendum to their paper.

Windaus and Lüttringhaus (1931) have more recently reported on the physical and physiological properties of the two definite crystalline substances D_1 and D_2. They showed that "calciferol" is identical with vitamin D_2. The marked toxicity which results from excessive doses is an actual property of the vitamins.

Bills and McDonald (1932) have synthesized vitamin D by treating ergosterol with nitrogen monoxide gas at a low temperature without the use of ultra-violet irradiation. The synthesis was carried out in solution with methyl alcohol, ether, and ethyl acetate in the absence of oxygen.

In their consideration of the fundamental nature of vitamin D action McGowan, Cunningham, and Auchinachie (1931) expressed the opinion that the probable mode of action of this vitamin in the cure and prevention of rickets, when the supply of phosphorus is inadequate, is the liberation of inorganic phosphate from the body lipins.

Methods of Assaying Vitamin D

By Use of the Line Test

The "line test" procedure for measuring antirachitic potency was developed in McCollum's laboratory. As originally employed young rats from 28 to 40 days old, weighing 55 to 60 gms., were kept on a rickets-producing diet until they showed evidences of well-developed rickets, at which time the metaphyses of the bones are generally free from calcium salts. The food to be tested was then fed for a given time, during which food consumption records were kept, since starvation would also cause calcium salts to be put down in the epiphyseal cartilage. At the end of the test feeding the animals were autopsied. The proximal end of the tibia was split with a scalpel, immersed in a 1% silver nitrate solution, exposed to light for several minutes, rinsed with distilled water and examined under the binocular microscope for a new deposition of calcium which, if present, would be stained black. If there is no calcification of the epiphyseal cartilage and little or none in the metaphysis, the "line test" is negative. When the test is positive the metaphyseal side of the epiphyseal cartilage shows a fairly broad linear deposit of calcium phosphate.

In their paper on the biochemical and spectroscopic studies on purified cholesterol, Bills, Honeywell, and MacNair (1928) presented a detailed discussion of the technique and the factors involved in using the line test for a quantitative assay of vitamin D. More recently Bills, Honeywell, Wirick, and Nussmeier (1931) carried their critical studies of the line test still further. They considered the test quite accurate.

By Use of the X-Ray

Poulsson and Lovenskiold (1928) prefer to X-ray the left knee joint at the beginning and at the end of the test-feeding period, and base their quantitative estimation of the antirachitic potency of the test material upon the changes noted in the radiographs. Knudson and Moore (1929) reported parallel studies of X-ray pictures with the "line test." They believe the "line test" to be more sensitive, but recommend skiagram observations to determine the proper time to begin the assay period and to follow its progress.

By Means of Intestinal pH

Zucker and Matzner (1924) observed that rats kept on a high-calcium, low-phosphorus rachitic diet developed decided fecal alkalinity, but the feces became acid again if an antirachitic factor was administered. Upon the basis of this work Jephcott and Bacharach (1926) described their method of measuring vitamin D in terms of intestinal pH. The test is rapid but has not proved generally satisfactory. Its reliability appears to be dependent upon a high concentration of vitamin D in the test material, cod liver oil, for example.

By a Study of the Composition of Blood

Howland and Kramer (1921) reported that the inorganic phosphorus of the blood serum is lowered in children during active rickets. Hess and Unger (1922) concluded that the lowering of inorganic phosphorus is not specific for rickets, but it generally occurs. Cavins (1924) reported 7 to 8.5 mgs. per 100 cc. as the normal inorganic phosphorus content of the blood serum of the rat, but this drops to around 3 mgs. per 100 cc. in a rachitic animal. Dutcher, Creighton, and Rothrock (1925) found that rats three weeks old with a normal blood phosphorus of 10 mgs. showed a drop to 1.6 mgs. per 100 cc. of blood after 21 days on a rickets-producing diet. Various other investigators have reported on studies of a similar nature.

Measuring Antirachitic Potency by Composition of Bone

Bethke, Steenbock, and Nelson (1923) reported about 45% ash in the femurs and humeri of rats 24 days old and weighing 45 to 60 gms.

At the age of 66 days the ash content had increased to 59%. They believed that the ash content should be determined on the basis of dry, ether-alcohol extracted bone and not on the " green bone " basis, because bones from animals on the same ration will show marked variations in lipoidal and fat content. On the basis of the inorganic phosphorus in the blood and the percentage of ash in the dry extracted femurs, Dutcher, Creighton and Rothrock (1925) set up certain standards for rats according to age, on normal, rachitic, and irradiated rachitic diets.

The percentage of calcium in dry bone or dry extracted bone has been used by Goldblatt and Soames (1923), by Chick and Roscoe (1926), and other English investigators to measure antirachitic potency.

Chick, Korenchevsky, and Roscoe (1926) suggested the use of a ratio expressed by $\frac{\text{Ash } (A)}{\text{organic residue } (R)}$ to express degree of calcification instead of percentage of calcium. The A/R value serves to magnify differences in bone composition.

Sherman and Stiebeling (1929) reported quantitative studies on responses to different intakes of vitamin D during the first three or four months of life. Their data indicate that the percentage of ash or of calcium in the fresh bone or in the dried alcohol-ether extracted bone, and the ratio of ash to organic residue of the dried extracted bone have all about the same degree of accuracy for measuring the degree of calcification. They recommend the use of the percentage of ash, or of calcium in the fresh bone.

In their investigation of the comparative ash content of the metaphyses and shafts of bones Hess, Berliner, and Weinstock (1931) found that osteoporosis of the shaft accompanies rickets in rats, and runs parallel with the loss of ash in the rachitic metaphyses, but in the process of healing, calcification of the metaphyses proceeds at a more rapid rate and the absolute amount of ash deposited in the metaphyses is greater than that deposited in the shaft. When laid down in the rachitic epiphysis a roentgenogram will show the deposition of as little as about one milligram of ash.

Gunderson (1931) has presented a preliminary report on a new and more rapid technique for assaying the antirachitic potency of foods, based on the percentage of ash in that fraction of bone which he designates as the proximal tibial enlargement, following the curative administration of antirachitic agent over a 14-day period. The proximal tibial enlargement includes proximal epiphysis and metaphyses with a minimal amount of diaphysis of the tibia, and constitutes from 20% to 18% of the length of the bone. The author has designed an apparatus which will permit quick and accurate sectioning of the tibia, and

is continuing his studies on this method of assay in the research laboratories, Quaker Oats Co.

Irradiated Foods

In a series of papers (1924–1925) Hess and Weinstock reported success in activating a number of products such as cottonseed oil, lettuce, the young wheat plant, spinach, patent flour, and other products. Boiling spinach for 30 minutes did not destroy its antirachitic value.

Steenbock and Black (1925) successfully activated lard, olive oil, cocoanut oil and fresh corn oil, whole milk and egg yolk, but rancid oils could not be activated. They reported that irradiation of egg yolk and whole milk increased their antirachitic value some 15 times. Steenbock and Daniels (1925) reported the activation of wheat, corn, hominy, cream of wheat, shredded wheat biscuit, rolled oats, milk, crude casein, egg yolk, butter, liver, cholesterol, phytosterol, and the unsaponifiable fraction of olive oil by ultra-violet irradiation. Hart et al. (1925) observed that eggs from hens which had been irradiated possessed an antirachitic value ten times that of eggs laid by non-irradiated hens. Numerous investigators in this country and abroad have reported upon their investigations of a similar character.

Activated olive oil is reported to have retained its potency for months in a stoppered bottle in the dark. Even saponification of an irradiated oil failed to destroy the active substance. Steenbock, Riising, Black, and Thomas (1929) found that the activity imparted to various cereal products was stable to autoclaving for 30 minutes at 15 pounds pressure followed by drying at 60° C. for a few days. Also storage for 16 months at 60° C. did not cause destruction, but the potency had decreased after storage for two years at 60° C., during which time the foods had become very rancid.

Supplee, Hanford, Dorcas, and Beck (1932) found that during the first few seconds of irradiation the rate of formation of vitamin D in fluid milk, exposed in the form of a moving film 0.4 mm. thick, was directly proportional to the amount of energy applied. Following this first period, however, the rate of formation was proportionately much slower. The maximum antirachitic value was imparted after approximately 2,500,000 ergs per cubic centimeter had been applied. The vitamin D potency of the irradiated milk was approximately 12 times that of the non-irradiated.

Experimental

Apparatus and Methods of Study

The researches reported in this paper were confined chiefly to a high grade soda cracker of the "Premium" type. In the earlier work the

familiar "line test" was made the basis of determining the antirachitic potency imparted by ultra-violet irradiation, but the later studies were carried out in accordance with the technique adopted by Steenbock which has already been briefly discussed.

A quartz mercury-vapor lamp (Hanovia, 110 volts, 5 amperes) was used as the source of ultra-violet irradiation. The total quantity of ultra-violet energy emitted by this lamp was measured from time to time by a fluorescent ultra-violet photometer of the type described by Anderson and Gordon (1928). This is a convenient instrument requiring no longer than 2 to 3 minutes to make a measurement. At a distance of 12 inches the total ultra-violet intensity of the particular lamp used in these studies was 94 ergs/per sec./mm.^{2}, a rather low value. A new lamp of this type emits 100 ergs or better/per sec./mm.^{2} at 20 inches. The readings remained quite constant throughout the course of this investigation, but they must be regarded as being only approximately accurate. An accurate instrument such as a Rentschler meter which measures ultra-violet radiation from 250 to 340 millimicrons would have been more desirable but was not available. The activation of ergosterol appears to proceed most satisfactorily with ultra-violet between the wave length limits of approximately 270 and 310 mμ. Longer rays are ineffective and rays shorter than about 270 mμ cause undesirable side reactions.

Supplee, Dorcas, and Hess (1932) have reported a precise study of the energy requirements for the antirachitic activation of milk under commercial conditions, and have confirmed their results with biological and clinical tests. They determined the antirachitic and calcifying potency of milk irradiated with definite amounts of energy from various sources.

In the studies made by us the wafers were irradiated at a distance of 12 inches for different time intervals ranging from 3 seconds up to 5 minutes. The shortening was irradiated in thin liquid layers, 2 to 3 millimeters in thickness, for periods of from 5 to 15 minutes. Samples of irradiated products were retained and examined from time to time in order to ascertain the effects of irradiation upon the induction period of oxidative rancidity.

The white rat was used as the experimental animal for determining the antirachitic value of the irradiated products. In all the work, litter mates were distributed over experiments so as to reduce error from litter variation. Each animal in a litter was placed on a different ration in an experimental series. If a given ration required five or more animals to secure the necessary data they were taken from as many litters, with but very few exceptions.

"Line Test" Studies

For the "line test" studies, young rats 28 days of age were placed on McCollums rachitic ration No. 4338 which is composed of:

	P.ct.
Soft wheat (low in phosphorus)	25
Yellow corn (pure bred, selection)	46
Gelatin	7
Wheat gluten	18
NaCl	1
$CaCO_3$	3

This is a high calcium, low phosphorus ration, containing as actually fed 1.25% of calcium and 0.283% of phosphorus, equivalent to the ration Ca:P::4.4:1. The wheat gluten was prepared from several dough batches containing 150 lbs. of high protein hard wheat flour. All gluten was washed free from starch before being dried and ground.

At the end of 21 days, the time interval required to produce rather severe rickets, each rat was again weighed and placed in an individual cage for a 6- to 7-day curative test period during which time it received the above rachitic ration which had been modified by the substitution (for a corresponding percentage of yellow corn) of 5% to 10% of the irradiated shortening, or of the wafers irradiated for the different time intervals. Accurate food consumption records were kept during the curative test period. The animals were then autopsied and examination made of the chest cavity, particularly the costochondral joints. The muscle tissue from the femur-tibia was also removed and the bones carefully split on the long axis. These bones were bleached in 5% formalin for several days, when the "line test" was made. The degree of staining with $AgNO_3$ showed the extent of calcification in the femur-tibia joint during the curative period with the modified rachitic ration.

The technique adopted by the University of Wisconsin, to be employed in determining a satisfactory degree of activation of cereal products by ultra-violet irradiation, differs in some of its details from certain methods now fairly well standardized and frequently used for the quantitative estimation of vitamin D.

The following are three good rations of practically equal value which may be employed for the experimental production of rickets in young rats:

Steenbock No. 2965	P.ct.
Yellow corn	76
Wheat gluten	20
$CaCO_3$	3
NaCl	1

McCollum No. 3143	P.ct.
Wheat	33
Yellow corn	33
Gelatin	15
Wheat gluten	15
$CaCO_3$	3
NaCl	1

McCollum No. 4338	P.ct.
Wheat	25
Yellow corn	46
Wheat gluten	18
Gelatin	7
$CaCO_3$	3
NaCl	1

Steenbock considers his ration, No. 2965, the most desirable because the higher percentage of yellow corn provides more of vitamin A, and the 20% of wheat gluten is superior to either of the mixtures of gluten and gelatin. He objects to the use of as much gelatin as is carried in ration No. 3143, since it may depress the appetite and thereby delay the onset of rickets. It may also cause diarrhea.

Ration No. 4338 more nearly approaches Steenbock's No. 2965, and is less subject to his criticism of No. 3143. As stated above ration No. 4338 is the one which was used for measuring the antirachitic potency by means of the "line test." For the production of rickets and histological studies following a curative feeding period ration No. 4338 would seem to be equally as satisfactory as No. 2965. In this connection reference may again be made to the recent paper by Bills, Honeywell, Wirick, and Nussmeier (1931). They used ration No. 3143 for their critical studies and believed that the extent to which any rachitic ration supports better growth than does No. 3143, will it likewise produce correspondingly poorer rickets.

For the best results from "line test" studies Steenbock believes the curative period should extend over 10 days, rather than the generally accepted time of from 5 to 7 days, since the young rat requires 2 or 3 days before it is able to start the recalcification process. Steenbock does not rely upon the "line test" as a measure of the degree of activation imparted to foods by irradiation. He employs the percentage of ash in the fat extracted bone. The "line test" has its greatest use in the assay of products which are quite potent in the D factor, such as cod liver oil.

To measure the activation of cereal products for human consumption Steenbock adopted the following ration as the basis of experimentation:

	P.ct.
Irradiated cereal product	76
Wheat gluten	14
$CaCO_3$	3
NaCl	1
Dried yeast	6

The degree of activation imparted by ultra-violet irradiation should be such that the above ration will give normal bone calcification (i.e., 50% ash or better on basis of fat-extracted bone) but when the 76% irradiated cereal product is diluted with itself in untreated form in the proportion of 3 (untreated) to 1 (treated), the degree of calcification will be less than normal—from 35 to 40%. This amount of activation is about the ideal to be approached according to Steenbock. He recommends dilutions of 1 to 3, 1 to 7, and 1 to 15, if necessary, in the exam-

ination of an irradiated product. Dilutions equivalent to the feeding of 5%, 10%, and 20% levels of the irradiated cereal product in the ration would accomplish the same practical ends and be a little more convenient to handle. The proper period of irradiation is generally a matter of a few seconds rather than minutes, but each product has to be put through a series of experiments before any conclusion can be reached. Over-irradiation is to be avoided from the standpoints of affecting the flavor and taste as well as to prevent giving foods medicinal properties.

Steenbock recommends that animals weighing from 55 to 60 gms. be selected (60 the ideal) and kept on the preventive series of diets for 5 weeks instead of 3 weeks, at which time the leg bones are fat-extracted and ashed. Four litters spread over a given series should give sufficient data for that particular series. Individual cages are used throughout the experimental period. Since litters differ in their susceptibility to rickets, even though they are from closely in-bred stock and are kept on the same diet and under the same environmental conditions generation after generation, it is important to observe the spread of each litter over any given series of experiments.

Procedure Used in Making Bone Ash Studies

Shortly after the experimental animals had been chloroformed the hind legs were removed, skinned, and then immersed in boiling water for 25 to 30 seconds in order that defleshing might be done easily and quickly. A scalpel was found convenient for removing from the bones the last portions of closely adhering flesh. The femur and tibia of each hind leg were kept intact, dried at 110° C. and crushed into small pieces with pliers. The crushed bony material of each leg was folded securely into an ashless filter paper, and fastened with a paper clip. A sizable collection of 150 to 200 samples was then extracted with 95% alcohol for 2 days in a Lloyd extractor, and this was followed with ether extraction in the same apparatus for 30 hours. The extracted bones were then ashed to constant weight in a muffle furnace at a temperature sufficiently high to yield a clean ash free from carbon. The material from each leg was handled separately and the results given in the tables represent the average figure per animal. It was believed that bone ash determinations would furnish more reliable quantitative data concerning the antirachitic potency of irradiated food products than would the use of the " line test," with its generally accepted method of expressing four degrees of healing.

Young rats 28 days of age were selected from parent stock maintained on a ration designed to produce experimental animals especially suitable for studies on rickets. This necessitated a diet low in the D

factor and one which was adequate in vitamin A, but not containing enough to cause appreciable storage of this vitamin in rats 28 days of age. The diet employed was a modified Steenbock ration consisting of the following components by weight:

	Parts
Yellow corn	65.50
Linseed oil meal	11.00
Casein	5.00
Meat scrap (containing some bone meal)	5.00
Alfalfa leaf meal	2.50
Salt	0.50
Calcium carbonate	0.50
Whole milk	liberal supply

Discussion

Potency of Irradiated Shortening Used in Crackers

Results of "Line Tests"

The antirachitic potency of crackers manufactured in the laboratory with irradiated shortening received some study. These wafers were made according to a representative formula using an hydrogenated shortening which had been irradiated for periods of 5, 10, and 15 minutes respectively. They were baked at 475° F. for 7 minutes. The usual time of baking in commercial practice is 5 minutes at 630° F. Experimental animals in which rickets had been induced in the prelimi-

TABLE I

Results of "Line Tests" for Curative Properties of Diets Fed for 6 Days in Which Crackers Were Substituted for Corn to the Extent of 10% of Rachitogenic Ration No. 4338

These crackers contained 14% of hydrogenated shortening which had been subjected to the irradiation treatment indicated before incorporating it in the cracker dough.

Ration	Rats of each sex	Initial weight of rats	Final weight of rats	Average daily food consumption	Degree of healing [1]
	No.	Gms.	Gms.	Gms.	
Control animals on ration No. 4338	4M3F [2]	47.9	74.0	—	[3]
213a. Non-irradiated shortening	2M3F	47.6	82.2	5.84	0.20
214a. Shortening irradiated 5 minutes	4M	45.7	75.7	5.90	2.75
215a. Shortening irradiated 10 minutes	1M3F	48.5	80.2	5.97	3.75
216a. Shortening irradiated 15 minutes	3M1F	43.5	73.7	5.27	3.25

[1] Four degrees of healing were recognized and recorded for individual animals ranging from 1+ to 4+, the latter representing practically complete healing.

[2] I.e., 4 males and 3 females.

[3] Severe rickets developed in these control animals.

nary feeding period were then placed on a diet in which yellow cornmeal, equivalent to 10% of the food mixture, was replaced with a like amount of the laboratory crackers. The control animals received a similar diet containing crackers baked with non-irradiated shortening.

The results secured with hydrogenated shortening are presented in Table I and indicate definitely that the irradiated shortening carried its antirachitic potency through the fermentation and baking processes into the baked crackers. The crackers from the non-irradiated, hydrogenated shortening showed no curative properties. Those made with shortening that had been irradiated for 5 minutes, effected a rather advanced healing of rickets during the 6-day test period. Crackers made with shortening irradiated for 10 minutes produced practically complete healing, and those prepared from shortening irradiated for 15 minutes were slightly less effective than was true in the instance of the 10 minutes irradiation. If this indicated decrease in potency is more real than apparent, there is a possibility that the longer period of irradiation may have caused some destruction of vitamin D.

TABLE II

RESULTS OF "LINE TESTS" FOR CURATIVE PROPERTIES OF DIETS IN WHICH LARD AND HYDROGENATED SHORTENING WERE FED AT THE LEVELS INDICATED FOR 6-DAY CURATIVE PERIODS

These fats had previously been subjected to the irradiation treatment indicated and replaced an equivalent quantity of corn in rachitogenic ration No. 4338.

Treatment accorded the fat	Rats of each sex	Initial weight of rats	Final weight of rats	Average daily food consumption	Degree of healing [1]
	No.	Gms.	Gms.	Gms.	
Lard Series					
10% non-irradiated	2M1F [2]	54.7	83.3	6.60	0.0
5% non- irradiated	1M1F	48.5	73.0	6.55	0.0
10% irradiated 5 minutes	1M1F	57.5	83.0	6.80	4.0
10% irradiated 10 minutes	2F	52.0	78.5	6.60	4.0
5% irradiated 5 minutes	2M	50.5	81.0	7.00	4.0
5% irradiated 10 minutes	1M1F	44.0	66.0	5.90	4.0
Hydrogenated Shortening Series					
10% non-irradiated	1M1F	54.0	69.5	5.45	0.0
10% irradiated 3 minutes	1M1F	55.0	75.5	6.05	4.0
10% irradiated 5 minutes	1M1F	54.5	69.5	5.50	3.5
10% irradiated 10 minutes	2M	48.5	74.0	5.10	4.0
5% irradiated 3 minutes	1M1F	52.0	65.5	5.15	3.0
5% irradiated 5 minutes	1M1F	51.5	67.0	5.65	4.0
5% irradiated 10 minutes	2M	55.0	62.5	5.80	3.0
Control Animals on Ration No. 4338					
	6M9F	50.8	71.3	—	[3]

[1], [2] See footnotes 1 and 2, Table I.

[3] Fairly severe to severe rickets developed in these control animals.

In another series of experiments shortening was warmed slightly, spread in layers 2 to 3 millimeters thick and subjected to ultra-violet irradiation for varying lengths of time ranging from 3 to 10 minutes. This shortening was then used to replace either 5% or 10% of corn in rachitogenic ration No. 4338. The degree of healing is recorded in Table II, together with other data respecting the weight of the rats and the food consumption. From these data it appears that 3 minutes of irradiation was sufficient to impart distinct antirachitic properties to both lard and hydrogenated shortening, and that this property was not substantially impaired by continuing the treatment through 10 minutes.

Potency of Irradiated Soda Crackers

"Line Test"

In the assays made by the "line test," soda crackers made with lard were irradiated for varying time periods on one and also on both sides, and then incorporated to the extent of 10% in the rachitic diet, being substituted for a like quantity of yellow corn. The curative picture presented by this method of investigation was clean cut and conclusive as shown by the data in Table III. The rachitic diet containing 10% of non-irradiated wafers produced no healing in rachitic animals when

TABLE III

Results of "Line Tests" for Curative Properties of Diets in Which Soda Crackers Made with Lard Were Subjected to the Irradiation Treatment Indicated and Substituted for 10% of the Corn in Rickets-Producing Ration No. 4338

The curative period extended over 6 days following a 21-day period on the rachitogenic diet.

Ration	Rats of each sex	Initial weight of rats	Final weight of rats	Average daily food consumption	Degree of healing [1]
	No.	Gms.	Gms.	Gms.	
Control animals on ration No. 4338	6M12F [2]	48.9	71.2	—	[3]
212a. Non-irradiated crackers	5M3F	44.0	71.6	5.01	0.0
221a. Irradiated 2 minutes one side	3M4F	52.0	89.7	5.07	3.1
209a. Irradiated 1 minute both sides	2M4F	45.3	66.3	4.68	3.0
222a. Irradiated 2 minutes both sides	3M2F	48.6	82.8	4.54	3.2
210a. Irradiated 2.5 minutes both sides	5M3F	48.2	67.5	5.44	3.0
211a. Irradiated 5 minutes both sides	4M3F	47.4	67.3	4.96	3.1

[1, 2] See footnotes 1 and 2, Table I.

[3] Fairly severe to severe rickets developed in these control animals.

lard was used as the shortening agent. The crackers irradiated on each side for 1 minute effected advanced healing in rachitic animals as did also those irradiated for the longer time periods.

In other series of experiments, data from which are not presented, the same type of crackers were irradiated on one side only for 2, 2.5, 5, 10, 15, and 20 minutes. These crackers were likewise used to replace 10% of corn in the rachitic ration. Curative results were produced in all instances. The crackers subjected to 2 minutes of irradiation on one side conferred a degree of antirachitic potency that produced advanced healing as definitely as did the longer periods. It was recognized that certain of the extended periods of irradiation would not prove commercially feasible, but it was desired to ascertain whether or not an abnormal extension of the irradiation period would cause a destruction of the antirachitic potency imparted in the early period of irradiation. This did not appear to be the case at this level. Lower levels were not investigated.

TABLE IV

RESULTS BY BONE ASH METHOD

White rats 28 days of age placed on experimental ration for 21 days, killed, the femora and tibia removed and their ash content determined. Soda crackers used in the numbered series replaced 10% of corn in rachitic ration No. 4338.

Ration	Animals of each sex	Average initial weight at 28 days	Average final weight at 49 days	Average daily food consumption	Ash in fat-free bone
	No.	Gms.	Gms.	Gms.	P.ct.
Modified Steenbock stock ration	5M3F [1]	52	Killed at 28 days	—	45.6 ± 0.23
Modified Steenbock stock ration	2M3F	51	130	8.5	53.2 ± 0.21
Rachitic ration No. 4338	4M5F	54	81	6.0	32.0 ± 0.45
226a. Crackers made with hydrogenated shortening	4M2F	53	78	5.7	33.6 ± 0.48
227a. Crackers made with lard	4M2F	53	80	5.9	32.1 ± 0.34
228a. Crackers made with hydrogenated shortening, irradiated 2 minutes on one side	4M4F	51	69	4.8	43.6 ± 0.52
229a. Crackers made with lard, irradiated 2 minutes on one side	5M1F	54	73	5.0	44.2 ± 0.69
230a. Same as 226a, irradiated 2 minutes on each side	4M3F	54	71	5.3	45.0 ± 0.31
231a. Same as 227a, irradiated 2 minutes on each side	4M2F	55	76	5.3	44.0 ± 0.22

[1] See footnote 2, Table I.

TABLE V

WHITE RATS 30 DAYS OF AGE PLACED ON EXPERIMENTAL RATION FOR 35 DAYS IN WHICH THE SODA CRACKERS WERE FED AT THE DESIGNATED PERCENTAGE OF THE ENTIRE RATION

Ration	Animals of each sex	Average initial weight at 30 days	Average final weight at 65 days	Average daily food consumption	Ash in fat-free bone
	No.	Gms.	Gms.	Gms.	P.ct.
Soda Crackers Containing Lard and Constituting 10% of Diet					
241a. Irradiated 5 seconds one side	2M4F [1]	56	122	8.3	52.1 ± 0.26
242a. Irradiated 10 seconds one side	2M2F	55	122	7.8	51.8 ± 0.25
243a. Irradiated 20 seconds one side	1M2F	55	133	8.4	53.3 ± 0.19
Soda Crackers Containing Lard and Constituting 20% of Diet					
237a. Irradiated 5 seconds one side	4M3F	58	122	8.2	51.2 ± 0.45
238a. Irradiated 10 seconds one side	1M4F	57	120	8.5	52.1 ± 0.39
239a. Irradiated 20 seconds one side	5F	57	119	8.3	53.5 ± 0.31
240a. Irradiated 30 seconds one side	3M3F	57	125	8.4	52.1 ± 0.41
Soda Crackers Containing Lard and Constituting 76% of Diet					
236a. Non-irradiated	4M5F	57	115	8.0	48.4 ± 0.66
232a. Irradiated 5 seconds one side	3M4F	60	119	8.2	50.8 ± 0.35
233a. Irradiated 10 seconds one side	3M3F	59	113	7.9	50.2 ± 0.35
234a. Irradiated 20 seconds one side	2M4F	57	117	8.2	51.6 ± 0.23
235a. Irradiated 30 seconds one side	2M4F	58	108	7.4	51.6 ± 0.31
Soda Crackers Containing Hydrogenated Shortening and Constituting 20% of Diet					
249a. Irradiated 5 seconds one side	2M1F	60	125	7.6	45.8 ± 0.65
250a. Irradiated 10 seconds one side	2M1F	61	142	8.5	49.4 ± 0.56
251a. Irradiated 20 seconds one side	1M1F	57	130	7.8	50.1 ± 0.50
Soda Crackers Containing Hydrogenated Shortening and Constituting 76% of Diet					
248a. Non-irradiated	2M2F	59	132	8.3	40.2 ± 0.33
244a. Irradiated 3 seconds one side	3M2F	59	144	8.4	49.3 ± 0.53
245a. Irradiated 5 seconds one side	2M2F	60	138	8.3	50.8 ± 0.65
246a. Irradiated 10 seconds one side	1M3F	60	121	7.5	50.9 ± 0.51
247a. Irradiated 20 seconds one side	1M3F	54	128	7.9	49.8 ± 0.67

[1] See footnote 2, Table I.

Bone Ash Method

Data involving the Steenbock technique for the commercial irradiation of cereal products are presented in Tables IV and V. These include the bone ash determinations, and the probable error of the mean has also been calculated for the results obtained in connection with the

Fig. 1

Fig. 2

Fig. 1. Photomicrograph of tibia of young rat with severe rickets from the control group (Table III).

Fig. 2. Photomicrograph illustrating the extent of healing produced by crackers irradiated 2½ minutes on each side, when these are consumed at a 10% level in ration No. 4338. This animal taken from lot 210a (Table III) consumed an average of 6.9 gms. of the ration during a 6-day curative period.

various rations and periods of irradiation. The results secured indicate that a period of irradiation of from 10 to 15 seconds during which the total ultra-violet intensity imposed by a mercury vapor lamp was 94 ergs per second per square millimeter imparted the degree of antirachitic potency which is considered desirable in accordance with the Steenbock technique for the commercial irradiation of cereal products.

Photographic and Radiographic Methods

In several instances typical bone (tibia) specimens were prepared for photographing. The fresh bones were first frozen in aqueous solution of gum acacia on the freezing microtome. A thin section was then

Fig. 3

Fig. 4

Fig. 3. Photomicrograph showing healing of rickets by feeding laboratory crackers at a 10% level, when these are made with hydrogenated shortening irradiated for 10 minutes. This degree of curing was effected in 6 days in a young rat from lot 215a (Table I). The average daily food consumption was 6.1 gms. during the curative period.

Fig. 4. Photomicrograph illustrating the extent of healing produced by feeding crackers irradiated 2 minutes on one side at a 10% level in rachitic ration No. 4338. This animal taken from lot 221a (Table III) consumed an average of 4.8 gms. of the ration per day over a 6-day curative period.

prepared by shaving off most of the bone on both sides of the center. This thin section was removed, thawed, washed free from the imbedding medium, treated with 1% silver nitrate solution, and then dehydrated with alcohol. The section was then covered with xylol in a flat bottom dish and photographed by transmitted light. Characteristic photomicrographs are included in Figures 1 to 6, and show graphically the extent of healing induced by irradiated crackers as contrasted with animals on the control rachitogenic diet (Fig. 1) and on a diet in which

10% of non-irradiated crackers replaced a like quantity of corn (Fig. 6).

Fig. 5

Fig. 6

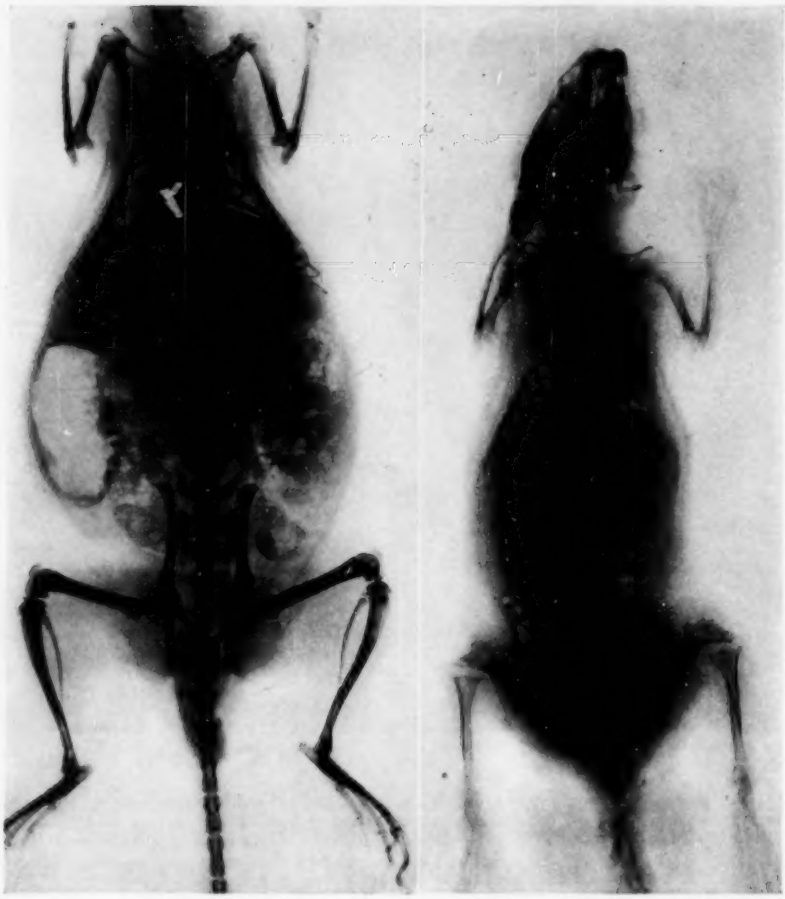
Fig. 5. Photomicrograph showing the extent of healing caused by the consumption of crackers irradiated for 2 minutes on both sides when these are incorporated at a 10% level in rachitic ration No. 4338. This animal taken from lot 222a (Table III) consumed an average of 5.8 gms. daily during the 6-day curative period.

Fig. 6. Photomicrograph showing the failure of non-irradiated crackers to cure rickets when these are fed at a 10% level in ration No. 4338. This animal taken from lot 212a (Table III) ate an average of 5.3 gms. per day during the 6-day curative period.

In other instances radiographs were made by the staff of the X-Ray Division of the University Hospital. Exposures of 10 seconds with 20 milliamperes, or 8 seconds with 30 milliamperes, were most successful. Four typical radiographs are shown in Figures 7 and 8. These illustrate the appearance of normal, and rachitic animals (Fig. 7), and of rachitic animals that have been cured by feeding irradiated crackers made with lard, and with hydrogenated shortening (Fig. 8).

Induction Period of Rancidity Development in Irradiated Crackers

From the inception of these experiments, it was recognized that the treatment of either shortening or baked crackers with ultra-violet light might tend to hasten the appearance of a rancid condition when the finished crackers were stored. It was also recognized that the type of



Rat No. 0 Rat No. 7

Fig. 7. Skiagraph of entire rats showing the relative effect of various rations on bone structure. Rat No. 0 from a lot fed the normal stock ration. Note dense bony structure.

Rat No. 7 fed non-irradiated crackers made with hydrogenated shortening at 10% level for 12-day period after having been on rachitogenic diet No. 4338 for 4 weeks. Note severe rachitic condition of the large bone of the hind legs.

Rat No. 1 Rat No. 5

Fig. 8. Skiagraph of entire rats showing the curative effect of irradiated crackers fed at 10% level for 12-day period after the animals had been on rachitogenic ration No. 4338 for 4 weeks.
Rat No. 1 from lot fed on soda crackers made with lard. The crackers had been irradiated 2 minutes on one side.
Rat No. 5 from lot fed on soda crackers made with hydrogenated shortening. The crackers had been irradiated 2 minutes on one side.

shortening used might have some bearing upon the effect of this treatment. With an hydrogenated shortening, irradiated for varying lengths of time on October 1, and examined on January 10, the shortening which was irradiated for 0 to 3 minutes was free from rancidity, while that irradiated for longer periods was rancid. These data are recorded in Table VI.

TABLE VI

HYDROGENATED SHORTENING IRRADIATED OCTOBER 1, IN FLUID LAYERS 2 TO 3 MILLIMETERS IN THICKNESS

Period of irradiation	Degree of rancidity on January 10
Non-irradiated	0[1]
3 minutes	0
5 minutes	++
10 minutes	++++
15 minutes	+

[1] The relative degree of rancidity is indicated by the plus sign +. One + represents detectable rancidity, ++ marked rancidity, +++ very marked rancidity, and so on.

TABLE VII

RESULTS OF OLFACTORY RANCIDITY TESTS OF SODA CRACKERS MADE WITH LARD AND SUBJECTED TO VARIOUS IRRADIATION TESTS

Soda Crackers Irradiated April 6 on Upper Side	
Period of irradiation	Degree of rancidity on July 16
Non-irradiated	0
5 seconds	++[1]
20 seconds	+++
30 seconds	+++
40 seconds	+++

Soda Crackers Baked July 20 and Irradiated August 1 on Upper Side	
Period of irradiation	Degree of rancidity on November 15
Non-irradiated	0
2 minutes	+++
5 minutes	+++
10 minutes	++++
15 minutes	++++
20 minutes	++++++

Soda Crackers Baked August 25 and Irradiated on September 30	
Period of irradiation	Degree of rancidity following January 10
Non-irradiated	0
1 minute, each side	++
2 minutes, each side	++
5 minutes, each side	+++

[1] See footnote 1, Table VI.

Crackers made with lard were then subjected to irradiation for varying periods up to 20 minutes. As shown by the data recorded in Table VII, even a few seconds of exposure to ultra-violet light served to accelerate the development of rancidity, while the untreated crackers were sweet and free from objectionable flavors even after 3½ months of storage at room temperature.

This effect of ultra-violet light upon the autoxidation of fats was similar to that observed by Holm, Greenbank, and Deysher (1927).

That hydrogenated shortening will likewise become rancid more rapidly if subjected to ultra-violet irradiation is evident from the data recorded in Table VIII. There was little difference between the lard and the hydrogenated shortening in that particular. It is thus apparent that the direct irradiation of foods rich in added shortening might introduce problems from the standpoint of the keeping qualities and marketability of the product.

TABLE VIII

OLFACTORY TESTS FOR RANCIDITY

The soda crackers made with lard were baked January 10, and were irradiated January 26. Those made with Crisco were baked January 18, and were also irradiated on January 26. Stored at ordinary room temperature.

Results with Lard as Shortening			
	Degree of Rancidity on		
Period of irradiation	March 6	April 23	May 22
Non-irradiated	0	0	+++[1]
2 minutes (upper side)	±	±	+++
2 minutes (each side)	++	++++	++++++
Results with Hydrogenated Shortening			
Period of irradiation	March 6	April 23	May 22
Non-irradiated	0	+	+++
2 minutes (upper side)	0	++	++++
2 minutes (each side)	+	++++	++++++

[1] See footnote 1, Table VI.

Summary

Studies have been made of the antirachitic potency imparted to soda crackers of the "Premium" grade when these are irradiated with a quartz mercury-vapor lamp of the general type (Hanovia, 110 volts–5 amperes) for varying periods of time at a distance of 12 inches. The quantity of ultra-violet energy emitted by this lamp at a distance of 1 foot was 94 ergs/per sec./per mm.[2]

Bone ash and "line test" methods of assay have both been used.

The influence of ultra-violet light in shortening the induction period

of oxidative rancidity was estimated qualitatively by means of odor intensity.

The data presented (Table V) show that some lards contain an appreciable amount of vitamin D, while hydrogenated shortening and certain other lards appear not to contain this dietary factor.

The technic adopted for the irradiation of soda crackers on a commercial scale apparently effected an antirachitic activation by an exposure for approximately 10 seconds to an intensity of ultra-violet radiation equivalent to 94 ergs/per sec./per mm.^{2} This figure is based on the bone ash data covering a 20% level of intake of crackers irradiated on one side, and applies to use of hydrogenated shortenings and lards containing no vitamin D. It is believed that the penetration of the ultra-violet rays into the biscuit is almost negligible.

Irradiation of soda crackers on one side only for periods much longer than 10 seconds showed no advantage from the standpoint of imparting antirachitic potency.

Laboratory crackers made with irradiated shortening carried their antirachitic potency through the fermentation and baking processes.

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A STUDY OF THE MARCH OF ACIDITY IN STORED FLOURS AND SOME CRITICAL REMARKS OF THE METHODS USED FOR THE DETERMINATION OF FLOUR ACIDITY

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Introduction

The problem of flour acidity and its changes during storage has attracted the attention of many investigators. Among these may be mentioned Jessen-Hansen (1911), Sharp (1924), and Bailey and Johnson (1924) who worked with changes in the hydrogen ion concentration of flours. Fifield and Bailey (1929) have studied the changes in titratable acidity, determined by different methods, and Johnson and Green (1931) have recently given a report on the nature of the acids formed in flour on aging.

The quantity of acid extracted from the flours depends upon the extraction media used. For instance, acidity as determined by the A. O. A. C. method does not increase as fast as the acidity determined by the Greek method. In a previous paper the author (1932) has suggested a method for the determination of flour acidity with 67% alcohol, since neither 85% alcohol nor water can dissolve all the acids present in the flour. It appeared of interest, therefore, to apply this method to a study of the change of acidity in flours in commercial storage, and to compare the results so obtained with the results obtained by some other methods used for determination of flour acidity.

Experimental

For the purpose, four flours were available. Three of these were rye flours, straight run to 68%, containing about 15% of wheat. Although from different mills, the flours were much alike and represented the type of rye flour used for breadmaking in Norway. Flour No. 4 was a patent wheat flour. All the flours came from Norwegian commercial mills and were analysed immediately after their arrival, the results obtained are given in Table I.

TABLE I

ANALYSIS OF FLOUR STUDIED

	Rye flour			Wheat flour
	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4
	P.ct.	P.ct.	P.ct.	P.ct.
Water	13.64	14.58	13.88	14.74
Ash	0.72	0.70	0.74	0.46
Protein	8.30	8.30	8.95	11.70
Fat (ether extracted)	1.18	1.30	1.30	1.34
Total P_2O_5	0.290	0.280	0.306	0.240

The samples, consisting of 30 kg. bags, were placed in the ordinary storage rooms of a large commercial bakery, where some thousand bags of flour usually were kept. The storage conditions, therefore, were identical with those used in industrial practice. It will be noted that the moisture contents of the flours did not change much during the examination period, which of course is an important item when interpreting the results.

During the first three months the temperature averaged 17° C. with relatively small deviations. During winter time it was reasonably lower, but since the increase in acidity was greatest in the beginning, temperature changes in the later period do not seem to be too important.

At intervals, samples were taken and acidity determined in extracts with 67% and 96% alcohol and ether. Acidity was also titrated in a fresh suspension of 10 gms. of flour in water.

Finally, suspensions were made with 50 gms. of flour in 150 cc. of water, plus the addition of 0.5% of formic aldehyde to prevent bacterial action. These suspensions were kept at 40° C. and portions equivalent to 10 gms. of flour were titrated after 24 and 48 hours. From the 48-hour suspensions, portions of the same size were diluted to 500 cc., filtered, and the quantity of P_2O_5 in the filtrate determined. The acidity of the aliquot was also determined. The results are given in Tables II to V. Acidity is expressed in terms of cc. N/1 alkali per 100 gms. of flour, and P_2O_5 in milligrams per 100 gms. of flour. The storage temperature is given in Table II only, since it was the same for all the flours.

Discussion

From the tables several deductions can be made. First, the total increase in acidity is approximately the same, within the same flour, whether the acidity is determined by extraction with ether, 67% or 96% alcohol. Even the rough titrations with the suspensions give the same results.

TABLE II

DATA OBTAINED FROM A STUDY OF FLOUR NO. 1

Weeks after milling	Acidity obtained by				Titer of suspension at end of		P_2O_5 dissolved in 48 hours	Titer at end of 48 hours in filtrate	Sum of acidity end of 48 hours[2]	Moisture in flour	Storage temperature
	Fresh suspension method	67% alcohol method	96% alcohol method	Ether extraction method	24 hours	48 hours					
	cc.[1]	cc.	cc.	cc.	cc.	cc.	mgs.	cc.	cc.	P.ct.	° C.
0	2.10	2.2	0.90	0.75	5.30	5.6	228	—	—	13.64	17
1	2.45	2.4	1.10	0.90	5.70	5.7	223	—	—	13.74	17
2	2.40	2.5	1.30	0.85	5.80	6.0	228	—	—	13.22	18
3	2.40	2.6	1.35	0.85	5.80	6.0	—	3.4	6.0	13.16	21
6	2.80	2.7	1.60	1.15	5.90	6.1	—	3.3	6.0	12.80	20
10	3.00	2.8	1.90	1.55	—	6.6	224	3.5	6.3	13.22	17
17	3.25	3.4	2.20	1.90	6.25	6.4	—	3.5	6.9	13.40	13
33	3.60	3.7	2.40	2.05	6.90	7.2	—	3.3	7.0	13.66	9
53	4.00	4.1	2.70	2.40	7.20	7.4	231	3.5	7.6	12.68	18
Total increase	1.9	1.9	1.8	1.65	—	1.8					

[1] N/1 alkali.
[2] Filtrate titer plus 67% alcohol titer.

TABLE III

DATA OBTAINED FROM A STUDY OF FLOUR NO. 2

Weeks after milling	Acidity obtained by				Titer of suspension at end of		P_2O_5 dissolved in 48 hours	Titer at end of 48 hours in filtrate	Sum of acidity end of 48 hours[2]	Moisture in flour
	Fresh suspension method	67% alcohol method	96% alcohol method	Ether extraction method	24 hours	48 hours				
	cc.[1]	cc.	cc.	cc.	cc.	cc.	mgs.	cc.	cc.	P.ct.
1	2.25	2.3	1.05	0.70	4.85	5.45	201	—	—	14.58
2	2.10	2.3	1.10	0.70	5.10	5.75	198	—	—	14.14
3	2.40	2.3	1.30	0.80	5.45	5.65	—	—	—	14.20
6	2.70	2.6	1.50	1.00	5.30	5.60	—	3.1	5.7	13.60
10	2.80	2.9	1.40	1.40	5.90	5.90	199	3.4	6.3	13.78
17	2.90	3.0	1.80	1.40	5.50	6.35	—	3.2	6.2	13.98
33	3.20	3.3	2.00	1.80	6.00	6.50	—	3.0	6.3	14.02
53	3.50	3.7	2.30	1.95	6.80	7.00	210	3.2	6.9	13.22
Total increase	1.25	1.4	1.25	1.25	—	1.55				

[1] N/1 alkali.
[2] Filtrate titer plus 67% alcohol.

TABLE IV

DATA OBTAINED FROM A STUDY OF FLOUR NO. 3

Weeks after milling	Acidity obtained by				Titer of suspension at end of		P_2O_5 dissolved in 48 hours	Titer at end of 48 hours in filtrate	Sum of acidity end of 48 hours[2]	Moisture in flour
	Fresh suspension method	67% alcohol method	96% alcohol method	Ether extraction method	24 hours	48 hours				
	cc.[1]	cc.	cc.	cc.	cc.	cc.	mgs.	cc.	cc.	P.ct.
1	2.40	2.2	1.05	0.75	5.45	6.0	224	—	—	13.88
2	2.25	2.3	1.10	0.80	5.80	6.2	223	—	—	13.18
3	2.45	2.5	1.20	0.80	5.90	—	—	—	—	13.52
6	2.65	2.7	1.55	1.20	5.90	6.3	—	3.4	6.1	13.12
10	2.95	3.0	1.80	1.60	6.20	6.4	218	3.6	6.6	13.64
17	3.05	3.1	2.00	1.70	6.15	6.7	—	3.6	6.7	13.12
33	3.55	3.3	2.40	2.05	6.85	7.2	—	3.3	6.6	13.78
53	3.95	3.8	2.70	2.30	7.20	7.6	233	3.5	7.3	13.00
Total increase	1.55	1.6	1.65	1.55	—	1.6				

[1] N/1 alkali.
[2] Filtrate titer plus 67% alcohol.

TABLE V

DATA OBTAINED FROM A STUDY OF FLOUR NO. 4

Weeks after milling	Acidity obtained by				Titer of suspension at end of		P_2O_5 dissolved in 48 hours	Titer at end of 48 hours in filtrate	Sum of acidity end of 48 hours[2]	Moisture in flour
	Fresh suspension method	67% alcohol method	96% alcohol method	Ether extraction method	24 hours	48 hours				
	cc.[1]	cc.	cc.	cc.	cc.	cc.	mgs.	cc.	cc.	P.ct.
1	1.95	2.0	0.8	0.70	3.6	3.8	106	—	—	14.74
2	1.75	2.1	0.7	0.70	3.4	3.8	112	—	—	13.56
3	1.90	2.2	1.0	0.75	3.8	3.8	—	2.1	4.3	14.32
6	2.05	2.1	1.3	0.90	4.1	3.8	—	1.9	4.0	13.88
10	2.40	2.5	1.3	1.25	3.8	4.0	105	2.0	4.5	14.28
17	2.55	2.7	1.6	1.25	3.9	—	—	1.9	—	13.76
33	2.40	2.9	1.6	1.55	4.3	4.2	—	2.0	4.9	13.98
53	2.75	3.3	2.1	1.85	4.9	4.7	115	2.0	5.3	13.66
Total increase	0.8	1.3	1.3	1.15	—	0.9				

[1] N/1 alkali.
[2] Filtrate titer plus 67% alcohol.

Second, the tables give a good opportunity to compare the quantity of acid extracted by the different media. Thus 96% alcohol consistently extracts more acid than does ether. This is in complete agreement with the findings of Herd and Amos (1930), that alcohol besides extracting some fat also extracts from flour some lipoids not soluble in ether. Evidently this will have an influence upon the quantity of fatty and similar acids which go into solution.

Again, 67% alcohol extracts a good deal more acid reacting material than does 96% alcohol or ether. As already mentioned by the author in a previous paper (1932), 67% alcohol dissolves from flour not only the fatty acids, but also some water-soluble acid constituents and some protein, which will bind some alkali because of its buffer action. Now the differences between the titers of the 96% alcohol, ether, and 67% alcohol extracts are practically constant during the examination period, i.e., the quantity of these acids does not increase, or, in other words, the fatty acids are alone responsible for the increase in flour acidity.

The quantity of P_2O_5 which goes into solution in the 48-hour suspensions deserves special attention. This was constant, within limits of error, during the whole examination period. The acidity of the filtrates is likewise a constant. It is the author's view that the P_2O_5 content is responsible for the acidity of watery extracts from flour. To test this assumption he has calculated the P_2O_5 as KH_2PO_4 in order to see how far this KH_2PO_4-titer agrees with the titer really found. The results of these calculations are given in Table VI.

TABLE VI

RELATION BETWEEN THE TITER FOUND IN FILTRATES FROM DILUTED 48-HOUR FLOUR SUSPENSIONS, AND THE ACIDITY CALCULATED FROM THE P_2O_5 IN SOLUTION INTO KH_2PO_4 AND EXPRESSED AS CC. OF STANDARD ALKALI

Flour number	Mean of titration of 48-hour suspension, filtrates expressed in cc. of normal alkali	Mean of P_2O_5 determinations, calculated as KH_2PO_4 and expressed in cc. of standard alkali
1	3.4	3.20
2	3.2	2.95
3	3.5	3.20
4	2.0	1.55

For flours Nos. 1 to 3, the calculated titer covers the experimental acidity from 92% to 94%; for wheat flour No. 4 it is covered to 78%. There seems to be some difference between rye and wheat flours in this respect, and it must be remembered that a much lower percentage of P_2O_5 goes into solution from wheat flour than from rye flour.

It is a question, then, how an increase in the A. O. A. C. acidity

shall be explained. In a study by Fifield and Bailey (1929), a comparison is made between the Greek and the A. O. A. C. acidity methods applied to the study of the development of acidity in flours in storage.

Both methods show a definite increase in acidity when the flours are aging, but the increase measured by the A. O. A. C. method is not as great as the increase determined by the Greek method. The rise in Greek acidity can rather easily be explained. It consists of an increasing amount of fatty acids plus a small and constant quantity of other acids. Since it contains the fatty acids as dominant factor, the Greek acidity can be compared with the results obtained with 96% and 67% alcohol, when it is calculated in terms of standard alkali.

The explanation of a rise in A. O. A. C. acidity, however, is more complicated. Fatty acids cannot give the effect, since they are insoluble in water. The titer of a watery extract from flour is greatly dominated by the phosphates, and these phosphates are not present in the dry flour, but are formed by a reaction between flour and water.

The author's examinations show that this reaction approaches a certain maximum, until a certain percentage of P_2O_5 is dissolved. In the flours examined, this percentage has been found to be practically constant during a year's storage.

Therefore, since an increase in the total quantity of phosphate cannot be found, the rise in acidity in watery flour extracts can only be explained in two ways: (1) There is formed an acid soluble both in alcohol and water, for instance, lactic acid, besides the fatty acids; and (2) a reaction between insoluble fatty acid and the phosphate, forming acid phosphate:

$$K_2HPO_4 + RCOOH \rightleftarrows KH_2PO_4 + RCOOK.$$

The presence of lactic acid in flours is not very likely, according to the examinations of Johnson and Green, and some experiments by the author go in the same direction.

Then, only the second possibility is left. The reaction scheme is reversible, and its direction will depend upon the strength of the acid and the hydrogen ion concentration of the solution. The author has not had an opportunity to make a closer examination of this theory, but some experiments have given promising results. A phosphate solution, of a concentration and pH usual for flour suspensions, is remarkably influenced both in pH and titer when shaken with pure oleic acid. This effect was definite, either the acid was used in free state or absorbed in wheat starch.

From these considerations the author comes to the conclusion that the A. O. A. C. method cannot be the correct method for the determination of flour acidity. It should seem preferable to determine the fatty

acids themselves by an alcohol extraction method, instead of examining their secondary effect on a phosphate solution.

There is still another point of interest concerning the filtrates from the 48-hour suspensions. The author has found the titer of these filtrates constant, or practically constant. Now, if it can be assumed that all acids present are titrated completely in a flour suspension, then the titer of a 48-hour suspension should consist of this constant "phosphate titer" plus the titer of the original flour, determined by titration of a fresh flour suspension or a 67% alcohol extract. In the tables these sums are given, and, when the inaccurateness of the suspension titrations is considered, they agree rather well with the actual acidity of the 48-hour suspensions.

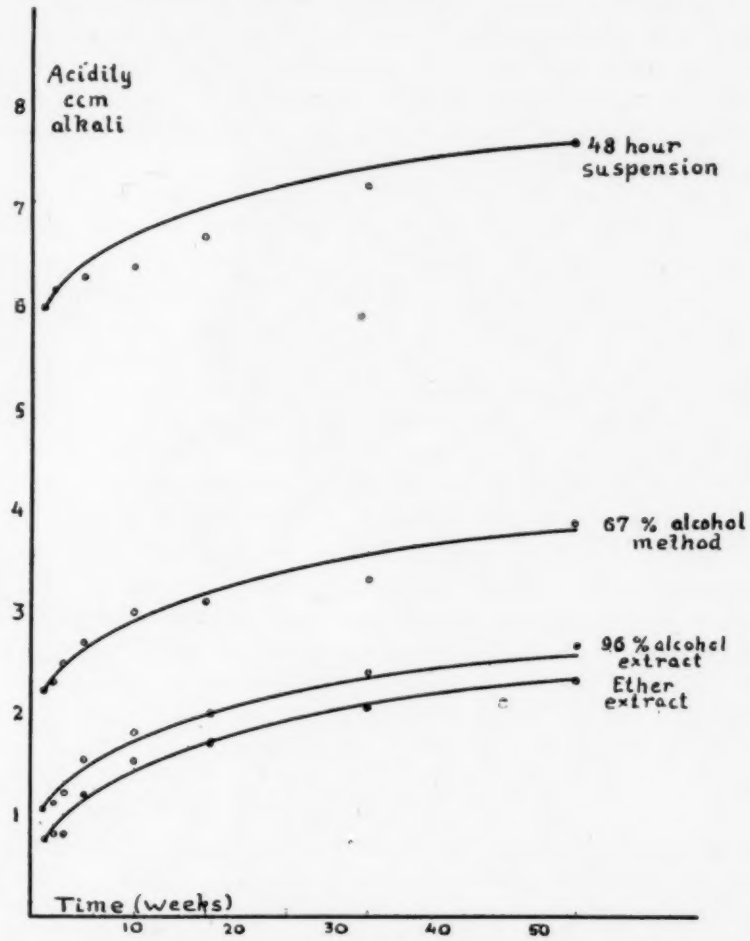

Fig. 1. Increase of acidity in flour No. 3 determined by different methods.

Finally, to give an impression of the picture of acidity development, the data for flour No. 3 are shown graphically in Figure 1. Since all the other flours give similar pictures, it was not considered necessary to reproduce all of them.

Most interesting is the regularity of the curves. The lines representing the different methods are perfectly alike. The rise in acidity is rapid in the beginning, and gets more slow the more acid is formed. Some of the curves given by Fifield and Bailey show the same tendency, although most of them have a rather irregular shape. It must be remembered, however, that their samples were rather small, and lost a good deal of their moisture when kept in thermostats. Most likely this may have had influence upon their results.

The author's material, the examination of four flours, may seem a small platform to build upon. But it seems permissible to suppose that when flours are stored under constant conditions, acidity will develop in a regular way. How far the acidity curves can be subject for mathematical analysis, it is too early to say. For this purpose, more material would be necessary, above all, contemporary determinations of titratable acidity and pH. The samples must be kept under constant conditions regarding temperature and moisture. Besides the ash of the flours, which is generally correlated to acidity, it seems necessary to accept their fat content as another determining factor.

Summary

Four 30-kg. samples, three rye flours, and one patent wheat flour, were stored under commercial conditions for a year. At intervals acidity was determined in ether, 96%, and 67% alcohol extracts, as well as in fresh suspensions of the flours. Other suspensions of the flours, with formic aldehyde added to prevent fermentation, were kept in thermostats at 40° C. and portions titrated after 24 and 48 hours. Portions from the 48-hour suspensions were diluted, filtered, and P_2O_5 and acidity determinations were made on the filtrates. From the results obtained, the following conclusions were drawn:

1. The total increase in acidity, within the same flour, is approximately the same by all the methods used.

2. 96% alcohol extracts more acid than ether, and 67% alcohol extracts more acid than 96% alcohol.

3. The differences between the methods used are practically constant during the examination period, i.e., the fatty acids are alone responsible for the increase in acidity.

4. The quantity of P_2O_5 and the titer of the filtrates from the 48-hour suspensions are constant. The P_2O_5, when calculated as KH_2PO_4, very nearly covers the titers of the extracts.

5. The Greek acidity method, using 85% alcohol, extracts the fatty acids, which are the dominant factor in flour acidity. Therefore, as far as a rise in acidity is concerned, results obtained by the Greek method are comparable with results obtained by other methods extracting the fatty acids, such as strong alcohol solutions and ether.

6. The author tries to explain the A. O. A. C. acidity as a secondary effect by a reaction between insoluble fatty acids and phosphates, forming acid phosphates. In his view, therefore, alcohol extraction methods should be more correct than the A. O. A. C. method for the determination of acidity.

7. A graphic picture of the development of acidity in flour studied is very regular. Indeed, when flours are stored under constant conditions, including the moisture, this is to be expected. For a mathematical study of these curves more material is necessary. Besides the ash, the fat contents of the flours should be recognized as a factor important for flour acidity.

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SOFT WINTER WHEAT STUDIES. I. THE SUITABILITY OF THE A. A. C. C. BASIC BAKING PROCEDURE FOR THE DETERMINATION OF STRENGTH

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In evaluating the quality of various soft winter wheat varieties grown under the Tri-State Soft Winter Wheat Improvement Association's program (Bayfield, 1932), the baking test among others is employed to obtain a measure of the strength of the various samples. Previous to the 1931 crop, large loaves were baked, each requiring 340 gms. of flour, 15 gms. of sugar, 6 gms. of salt, 15 gms. of yeast, and water (tap), varied in amount to suit the requirements of the flour concerned. A fixed fermentation period (50, 20, and 10-minute periods between punches, followed by a 5-minute rest period before panning) at 80° F. was used. Similarly, the proofing period of 50 minutes at 95° F. was uniform in all cases. The baking temperature was 220° C. for 35 minutes. This procedure produced satisfactory loaves of bread.

Commencing with the 1931 crop, it was decided to employ the basic baking procedure of the A. A. C. C. as closely as possible considering available laboratory equipment. A small KitchenAide (2-quart bowl) mixer was used for mixing the doughs. This machine employs a single paddle for mixing the ingredients and with an experienced operator proves reasonably satisfactory. Regulation high baking pans were used (Blish 1928, Harrel 1929).

The first difficulty experienced with the basic formula was that of absorption. The specified 58% proved too high for the majority of soft winter wheat flours. This was also the experience of Whiting (Blish, 1928a) in working with commercial flours of similar characteristics. Accordingly, with the experimentally milled flours under investigation, it was considered advisable to use a variable absorption. This also seemed desirable in view of the wide range in protein found to exist in the flour samples. This range in protein amounted to 10.3% (5.5% to 15.8%) when calculated to a 15% moisture basis.

The basic procedure of the A. A. C. C. calls for a fermentation

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period of 105 minutes to the first punch, followed by a second punch 50 minutes after the first one. The dough is molded and placed in the pan 25 minutes after the second punch. This total of 3 hours of fermentation is carried out at 30° C. ± 0.5° and with a relative humidity of not less than 75%. The proofing period is 55 minutes and under the same temperature conditions as for the fermentation period. The baking temperature is 230° C. ± 5° for 25 minutes. It was found in this work that 30 minutes produced somewhat better results; accordingly, a 30-minute period was used throughout.

It was realized that the adoption of the basic procedure meant a rather radical change, particularly in so far as fermentation conditions were concerned. Fermentation studies reported upon by Shiple (1931) and Bayfield (1931) indicated that with 105 minutes elapsing before the first punch, many doughs would become too old. This difficulty, however, is an inherent disadvantage of any fixed procedure which necessarily can not be adapted to meet the individual requirements of each flour.

A series of 72 samples was baked in duplicate, the duplicate bakings being made on a different day from that of the first baking. A small percentage of the duplicate bakes differed from the first bake by more than 20 cc. in loaf volume and in these cases the bakes were repeated. In general appearance these loaves proved very disappointing. They were pale in crust color; a high percentage having either shell or split tops. The internal texture was generally poor and the color bad. The loaves gave many indications of lack of sufficient sugar to withstand the long fermentation period. Accordingly a number of new bakes were made using different amounts of sugar. From these it appeared that the use of 3.5 gms. of sugar largely remedied the trouble. With the use of this amount of sugar the crust color became much better and the internal texture and color became reasonably good.

The entire series of 72 samples was then re-baked in duplicate as before excepting that 3.5 gms. of sugar was used. Compared with the first series of tests, with the increase in sugar 51 of the 72 samples gave positive increases in loaf volume, 5 samples did not change, and 16 gave smaller volumes. The average loaf volume for all samples with 2.5 gms. of sugar was 421 cc., while with 3.5 gms. the volume was 443 cc., an average increase of 22 cc. These results corroborate the statement of Whiting (Blish, 1928a) that possibly the percentage of sugar should be increased. Whiting also considered that a fermentation period of 3 hours was too long for soft wheat flours.

To further test the effect of the additional sugar on loaf volume, correlations between loaf volume by either procedure and percentage crude protein in flour were determined. With the small dosage of

sugar the coefficient equaled $-.1836 \pm .0768$ whereas with the use of the larger quantity of sugar it increased to $+.2410 \pm .0749$. While these coefficients were small yet they gave additional demonstration that the basic formula did not provide enough sugar, at least in so far as these particular samples were concerned. At the time it seemed preferable, to the authors, to add more sugar to the formula rather than to change the fermentation periods called for in the basic procedure. Accordingly 3.5 gms. of sugar was used as standard for bakes reported in this paper.

In the above mentioned 72 flour samples there were a few which possessed higher protein contents than are normal for the varieties concerned. The protein content for all 72 samples averaged 10.18%. These high protein flours (12% to 13%) did not produce loaf volumes in keeping with their protein contents. The ash contents of these high protein samples were within the same range as the remainder of the group (0.4% to 0.5%) so this was not the cause of the discrepancy.

Larmour and MacLeod (1929, 1929a) conclude that it is highly advisable to use the bromate differential test in testing the quality of wheat by the experimental milling and baking procedure. Larmour (1930) believes that the bromate differential test gives a more accurate measure of quality than the basic procedure in so far as hard red spring wheats are concerned. Larmour and MacLeod (1929, 1929a) carried out their work as part of the extensive program of the Associate Committee on Grain Research, National Research Council of Canada. Since then the results of a great deal of work by this committee have been published. The baking procedures followed by the members of this committee are essentially the same as recommended by the A. A. C. C., the principal differences being the type of pan used (low type), and a variable instead of fixed absorption. Committee papers such as those mentioned and the more recent results of Larmour (1931), Geddes, Malloch, and Larmour (1932), and Malloch, Geddes and Larmour (1932) indicate that the basic formula does not give as true a relationship between loaf volume and protein content as the bromate differential test. Papers by Harris (1930, 1931) also show that protein content and loaf volume are more highly correlated when the bromate differential test is employed than is the case when the basic procedure is used. On the other hand, Mangels and Stoa (1931) state that the bromate modification of the baking test apparently does not have much value for classifying spring wheat varieties.

One of the main projects under the Tri-State Soft Wheat Improvement Association's program (Bayfield, 1932) is that of zoning the three states of Ohio, Indiana, and Michigan, in an attempt to standardize the wheat originating in each zone.

To group the various samples according to strength it is essential that a true estimate of quality result from the baking tests. As it was shown earlier in this paper that flours from certain high protein wheat samples did not give as large a loaf volume as would be expected from their protein content, it was decided to re-bake the entire group of samples from the 1931 crop and use the bromate differential test, Supplement C, A. A. C. C., in this second series of baking tests. Unfortunately, all the flours from the original 72 samples which were tested by the basic procedure of the A. A. C. C. were not available and it was necessary to select other varieties of wheat of variable characteristics for this purpose. Ten varieties of wheat, therefore, each grown at a different Ohio location were selected for this study.

This variation in location gave a considerable range in protein content due to changes in growing environments. The 10 varieties included a range in strength from the "strong" Red Rock to the "weak" American Banner with Kharkov as a hard winter type for comparison. It should be mentioned regarding this latter variety that under Ohio conditions it has been relatively moderate in strength. The threshed grain lost much of the typical appearance expected in a hard red winter wheat. The 10 varieties were: Trumbull, Nabob, Fulhio, Red Rock, American Banner, Berkeley Rock, Michigan Amber, Kharkov, Fultz, and Gladden. All these varieties, excepting American Banner, are red wheats.

Table I, grouped according to location, gives the average results obtained from the different varieties. All protein, ash, and absorption figures are corrected to a 15% moisture basis throughout. It will be observed that the change in location produced considerable variation in the wheats.

TABLE I

AVERAGE ANALYTICAL AND BAKING DATA OBTAINED FOR 10 VARITIES WHEN GROWN AT DIFFERENT OHIO LOCATIONS, 1931

Sample numbers	Test weight	Protein (wheat)	Protein (flour)	Ash (flour)	Absorption	Loaf volume (basic)	Loaf volume (bromate)	Response to bromate
	Lbs.	P.ct.	P.ct.	P.ct.	P.ct.	Cc.	Cc.	Cc.
911–920	58.9	8.82	7.37	0.49	55.3	417.2	397.4	— 19.8
921–930	59.4	9.10	7.56	.48	54.8	432.8	422.8	— 10.0
931–940	59.4	9.09	7.82	.47	54.9	440.7[1]	440.9[1]	+ 0.2[1]
941–950	59.7	10.10	8.74	.48	55.3	459.9	449.2	— 10.7
971–980	60.1	9.95	8.59	.50	55.3	442.9	473.6	+ 30.7
981–990	57.0	9.86	8.26	.49	55.1	444.3	466.9	+ 22.6
991–1000	58.1	9.74	8.28	.52	55.0	421.6	442.2	+ 20.6
1001–1010	55.8	10.71	9.28	.57	55.8	465.9	490.1	+ 24.2
1011–1020	54.5	11.16	9.64	.56	54.9	453.7	502.9	+ 49.2
1021–1030	52.5	12.74	11.02	.57	55.9	474.8	541.3	+ 66.5

[1] Berkeley Rock missing, average of 9 varieties only.

From Table I it can be seen that there was a decided trend for an increase in loaf volume with bromate as the protein content increased. Also it may be observed that samples 911 to 950 appear to behave differently from the remainder. These samples (911 to 950) were grown on different soil types but under the *same* climatic conditions, whereas the remainder were grown under conditions where climate was the main variable. Unless environment produces some change

TABLE II

ANALYTICAL AND BAKING DATA ARRANGED ACCORDING TO VARIETY AND CONSIDERING THE ENVIRONMENT AFFECTING THE WHEAT

Variety		Test weight	Protein (wheat)	Protein (flour)	Ash (flour)	Absorption	Loaf volume (basic)	Loaf volume (bromate)	Response to bromate
		Lbs.	P.ct.	P.ct.	P.ct.	P.ct.	Cc.	Cc.	Cc.
Trumbull	A[1]	60.0	9.8	8.5	0.460	54.6	450.5	434.2	— 16.3
	B	56.7	10.9	9.3	.484	54.3	470.3	498.2	+ 27.9
	C	58.0	10.5	9.0	.475	54.4	462.4	472.6	+ 10.2
Red Rock	A	59.5	9.4	8.0	.490	55.1	455.5	441.7	— 13.8
	B	56.5	10.8	9.4	.549	55.8	463.7	490.3	+ 26.6
	C	57.7	10.4	8.9	.525	55.5	460.4	470.9	+ 10.5
Fulhio	A	60.0	9.6	8.3	.455	55.9	452.2	438.5	— 13.7
	B	56.5	11.0	9.6	.542	55.5	461.3	481.0	+ 19.7
	C	57.9	10.4	9.1	.508	55.7	457.7	463.9	+ 6.2
Kharkov	A	59.3	9.4	7.9	.490	53.9	438.7	409.7	— 29.0
	B	56.5	10.9	9.5	.579	55.6	459.8	495.8	+ 36.0
	C	57.6	10.3	8.9	.544	54.9	451.4	461.4	+ 10.0
Michigan Amber	A	58.9	9.5	8.1	.487	55.5	424.7	420.5	— 4.2
	B	56.1	11.2	9.6	.551	56.1	454.0	474.3	+ 20.3
	C	57.2	10.5	9.0	.525	55.8	442.3	452.8	+ 10.5
Gladden	A	59.6	8.6	7.2	.462	53.8	431.5	427.5	— 4.0
	B	56.8	10.3	8.6	.490	53.9	446.5	484.0	+ 37.5
	C	57.9	9.6	8.0	.479	53.9	440.5	461.4	+ 20.9
Fultz	A	59.3	9.3	7.9	.480	55.2	430.0	438.0	+ 8.0
	B	55.8	10.8	9.2	.515	55.0	446.5	500.2	+ 53.7
	C	57.2	10.2	8.7	.501	55.1	439.9	475.3	+ 35.4
Berkeley Rock	A	60.0	9.1	7.8	.585	58.4	431.7[2]	427.3[2]	— 3.4[2]
	B	56.6	10.9	9.6	.594	59.0	441.5	486.5	+ 45.0
	C	58.0	10.2	8.9	.590	58.8	438.2[3]	466.8[3]	+ 28.6[3]
American Banner	A	57.3	8.9	7.3	.459	53.6	429.2	417.2	— 12.0
	B	54.7	10.0	8.5	.535	53.9	435.0	481.2	+ 46.2
	C	55.7	9.5	8.0	.505	53.8	432.7	455.6	+ 22.9
Nabob	A	59.5	8.9	7.6	.444	54.6	430.0	417.5	— 12.5
	B	57.2	9.9	8.4	.524	54.3	426.7	470.3	+ 43.6
	C	58.1	9.5	8.1	.492	54.4	428.0	449.2	+ 21.2

[1] A = averages from samples numbering 911 to 950 inclusive (Table I). B = averages from samples numbering 971 to 1030 inclusive (Table I). C = averages from all 10 locations.
[2] Only 3 samples baked.
[3] Only 9 samples baked.

which affects the response to bromate, it is difficult to understand why the protein in samples 941–950 gave a negative response, whereas protein in the same varieties included in samples 971–1000 gave positive responses. At this time, however, no answer seems apparent regarding this question of the effect of environment upon the bromate differential test. It can be mentioned though that all of these wheat samples were sound and uninjured.

Table II presents the analytical and baking data for the samples when arranged according to variety. All 10 locations are averaged in the C groups and the varieties are presented in decreasing order of strength as determined by the basic baking method (C groupings). The A groups include the samples produced under the same climatic conditions. With this arrangement we see that all varieties excepting Fultz gave negative responses to bromate. The B groups consist of those samples produced under conditions with a considerable variation in the climate. Positive responses to bromate were the rule with these groups.

Examination of the data in Table II shows the test weight per bushel the highest, and the protein, ash, and loaf volumes the lowest for the A groups; while the opposite holds true for the B groups. As might be expected from the C groups, this arrangement gives results, the magnitudes of which are above the A grouping and below the B grouping. By means of these grouping arrangements we may see whether the same varieties hold the same relative strength ranking at different protein content levels. We may also see how the bromate bakes compare with those made by the basic method.

TABLE III

STRENGTH RATING ACCORDING TO GROUP CLASSIFICATION

Variety	A Group			B Group			C Group		
	Protein (wheat)	Basic bakes	Bromate bakes	Protein (wheat)	Basic bakes	Bromate bakes	Protein (wheat)	Basic bakes	Bromate bakes
Trumbull	1	3	4	3	1	2	1	1	2
Red Rock	4	1	1	6	2	4	3	2	3
Fulhio	2	2	2	2	3	8	3	3	5
Kharkov	4	4	10	3	4	3	5	4	6
Michigan Amber	3	10	7	1	5	9	1	5	9
Gladden	10	6	5	8	6	6	8	6	6
Fultz	6	7	3	6	6	1	6	7	1
Berkeley Rock	7	5	6	3	8	5	6	8	4
American Banner	8	9	9	9	9	7	9	9	8
Nabob	8	7	8	10	10	10	9	10	10

Table III shows that the varieties rank somewhat differently depending upon the baking procedure used and upon the amount of protein. It appears that the order of rank between the protein levels agreed reasonably well, although there were some exceptions. Such exceptions, however, may be expected when dealing with relatively small numbers of samples grown under varying environments. The baking procedure appears more important than the effect of protein quantity. The question then arises, does or does not the basic baking procedure give the most logical results?

From previous experience with these varieties we would expect Red Rock to be the strongest. Fultz normally is stronger than Gladden or Michigan Amber. Berkeley Rock is variable in its strength due to its being difficult to mill; as a result it usually posseses too high an ash content, which in turn often reduces its baking values. Michigan Amber frequently produces high ash flours also, and sometimes lacks strength for this reason. Usually it does not possess as high a relative amount of protein as is the case in these 1931 samples. Considering all factors, it would appear as though the bromate strength rankings agree more closely with that which would be normally expected than is true in the case of the basic bake strength rankings.

A few statements regarding the effect of bromate on the internal characteristics of the loaf may be of interest at this point. Comparison of the averages obtained from the 99 samples indicate a small decrease in score for grain (from 96.9 to 96.5). Similarly, the texture score decreased slightly (from 97.1 to 96.4). The use of bromate also reduced the color score somewhat (from 97.0 to 95.5). On the other hand, the color of loaf crust was improved by bromating; this score being raised from 6.2 to 7.5, out of a perfect possible score of 10. In view of the difficulty of scoring accurately and the small average differences obtained, it did not seem necessary to give the various variety scorings in detail. All varieties behaved very much alike in their response to bromate in so far as internal characteristics were concerned.

To obtain some further light on the question of the desirability of the two methods of baking, a few correlation coefficients were calculated on the whole group of samples. As complete baking data were available for only 99 of the 100 samples, only the 99 for which complete data were available were used in this study. Table IV gives the statistical coefficients obtained. In this table some coefficients are also given regarding the " fermentation index A " determination of strength. This index was obtained by measuring in square centimeters the area beneath a curve obtained by plotting volume against time during the rise of a 100-gm. dough (100 gms. of flour) in a Chidlow expansimeter jar. In the case of these 1931 samples, half of a 200-gm. dough (200 gms. of

flour) was used for the expansimeter test while the other half was used in the basic baking test.

TABLE IV

THE RELATIONSHIP BETWEEN SOME MEASURES OF QUALITY

Coefficient between	Per cent crude protein (wheat)	Per cent crude protein (flour)	Loaf volume (basic method)	Loaf volume (bromate method)
Loaf volume (basic)	$+.6455 \pm .0395$	$+.6397 \pm .0400$		
Loaf volume (bromate)	$+.7895 \pm .0255$	$+.7707 \pm .0275$		
Fermentation Index "A"	$+.5403 \pm .0480$	$+.5211 \pm .0494$	$+.4563 \pm .0537$	$+.5052 \pm .0505$
Absorption (per cent)		$+.3638 \pm .0588$		
Test weight per bushel	$-.7223 \pm .0324$			

From the above it will be seen that the use of bromate has increased the positive relationship between loaf volume and crude protein. From these results it would appear that bromate should be used in baking tests made with experimentally milled soft winter wheat flours. Apparently the conclusions regarding the bromate differential test stated by Larmour and MacLeod (1929) and by Blish and Sandstedt (1927) hold, in some degree, at least, for the weaker Ohio wheats as well as for the stronger wheats ordinarily used by these investigators. While the size of the coefficients between protein and loaf volume obtained in this study are not as large as many of those obtained by such Canadian workers as Larmour, Geddes, Harris, or Malloch, yet those obtained are quite significant. The authors believe that proper modification of the baking test should still further increase the magnitudes of these relationships.

Conclusions

Work with experimentally milled soft winter wheat flours of the 1931 crop indicated that the A. A. C. C. basic baking procedure was not well adapted for determining the strength of these flours. The absorption basis of 58% was too high for flours made from ordinary Ohio wheats. It was also found that the 2.5 gms. of sugar used in the basic formula was insufficient for these experimentally milled flours. Very probably more sugar could be used than the 3.5 gms. employed in these studies. In some cases, a deficiency in diastatic activity may mask the true strength of the gluten.

For differentiating between the strength of different varieties of soft winter wheats these studies indicate that the bromate differential test may be employed to advantage.

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BIOLOGICAL METHOD FOR THE DETERMINATION OF DIFFERENT SUGARS IN STARCH DEGRADATION PRODUCTS

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Introduction

The estimation of sugars by fermentation methods offers possibilities which are of interest especially in those cases where strictly chemical methods fail. Many investigators have shown that pure cultures of yeasts and other organisms exhibit a pronounced selectivity in respect to their fermentative action upon certain sugars.

Pure cultures of yeasts containing definite enzymes have been used successfully by Davis and Daish (1913) to estimate the sugars present in plant extracts. Nanji and Beazeley (1926) employed pure cultures of yeasts for the analysis of mixtures of starch sugars. McLachlan (1928) examined various methods for the determination of sugars in starch degradation products and concluded, as did the other investigators mentioned, that the method of selective fermentation by pure cultures of yeasts was the most satisfactory.

In carrying out the determinations with the fermentation methods referred to above, small inoculations of pure cultures were used, and the time for complete fermentation extended upwards to several weeks. The change in copper reducing power, polarization, or total solids after complete fermentation was used as a measure of the sugar present. The time required for complete fermentation is, under some circumstances, a serious disadvantage.

Slator (1920) by measuring the amount of carbon dioxide evolved, utilized the fermentation reaction itself for the estimation of sugars.

In investigating the rate of enzyme hydrolysis of soluble starch, Schultz and Landis (1932) used bakers' yeast, and measured the rate of maltose formation by a fermentation method in which the conditions were such that a stoichiometrical relationship exists between the amount of carbon dioxide evolved and the amount of maltose formed.

Description of Method

In the present paper a method of selective fermentation has been employed for the determination of dextrose or laevulose, sucrose, and

maltose in the presence of each other. The method involves the use of, (1) a pure culture of an air borne organism which has been classified by us as a mycoderma and which ferments dextrose or laevulose but does not ferment sucrose or maltose, (2) an active invertase preparation, and (3) fresh bakers' yeast.

Dextrose or laevulose was determined by collecting and measuring the volume of carbon dioxide produced by complete fermentation with the mycoderma organism. At the completion of this fermentation, a sugar-free and maltase-free invertase preparation was added to the reacting solution, and the volume of carbon dioxide then evolved is taken as the measure of the sucrose present. The total fermentable sugars was determined by fermentation with bakers' yeast. The difference between the carbon dioxide thus obtained from the total sugars, and that which is equivalent to the sum of dextrose and sucrose, was calculated to maltose.

The relationship between the volume of carbon dioxide measured and the amount of sugar present was experimentally determined for dextrose, sucrose, and maltose, with both organisms used. The relationships thus found with both organisms are fairly constant for a particular sugar when the total fermentable sugars present in the test solution does not exceed about four grams.

In further experiments it was found that under the conditions laid down for carrying out the fermentations, maltose and sucrose were absolutely unfermentable by the mycoderma organism. Full recovery of these sugars, as measured by the carbon dioxide evolved, could be obtained by the addition of bakers' yeast several hours subsequent to the addition of the mycoderma organism. Furthermore, the concentration of alcohol in the reacting solution was found to be without measurable effect upon the solubility of carbon dioxide in the reacting solution when not more than four grams of sugar are fermented.

Apparatus

The apparatus used was that described by Schultz and Landis (1932). Six, 200 cc. wide-mouthed reaction bottles are attached by means of spring fastening clamps to a cradle of strap iron. The cradle is continuously rocked through an arc of about 20 degrees in an electrically controlled thermostat by a small motor at a rate of about 120 oscillations per minute. Six, 1-liter gasometers or gas burets are attached by flexible connections to their respective bottles. The water expelled by the evolved carbon dioxide is collected in liter wash bottles which also serve as leveling bulbs when taking readings. A layer of kerosene serves to minimize solution of carbon dioxide in the water. While such a layer is not completely impervious to the gas, it was found in a blank experiment that the rate of solution was negligible.

Details of Method

Reagents

(1) A buffer solution of 100 gms. of potassium citrate, 20 gms. of citric acid, and 20 gms. of primary ammonium phosphate per liter, sterilized by boiling, has been found satisfactory as it gives very closely the optimum pH for fermentation. If not more than 4 gms. of sugar is fermented from one test solution, the use of this buffer in the amounts recommended has been found to hold the pH of the solution within a range wherein differences in solubility of CO_2 are so minimized as to be negligible; (2) a quantity of the mycoderma organism; (3) a quantity of fresh bakers' yeast; and (4) pure sugar samples for standard reference.

Procedure

1. *Determination of Dextrose and Laevulose.* 1 gm. of dextrose[1] is weighed into the reaction bottle, and 25 cc. of buffer solution added. 5 gms. of the mycoderma organism is weighed into a beaker, creamed up with distilled water, and washed into the reaction bottle using a total of 75 cc. of water. The reaction bottle is then placed in the cradle, and connected to the gasometer. Shaking is commenced and continued until the initial charge of dextrose is completely fermented out, as shown by the constancy of successive readings on the gasometer over two 15-minute periods. The shaking is then stopped, the gasometers reset to zero, a weighed sample of the material to be analyzed is added, and the zero reading is noted. Shaking is resumed and continued until fermentation is complete as indicated by the constancy of the gasometer readings for two successive 15-minute periods. The volume of CO_2 collected, divided by the volume of CO_2 obtained from 1 gm. of pure standard dextrose in a parallel experiment, equals the number of grams of dextrose or laevulose present in the sample taken.

2. *Determination of Sucrose.* After the fermentation above is complete, shaking is stopped, the gasometer is reset to zero, and 2 cc. of a 5% solution of a commercial invertase preparation is added, the zero reading noted, and shaking is resumed until the sucrose is completely fermented as indicated by a constant reading. The volume of CO_2 thus obtained, divided by the volume of CO_2 obtained from 1 gm. of sucrose in a parallel experiment, equals the number of grams of sucrose present.

3. *Total Fermentable Sugars.* In this instance 3 gms. of fresh bakers' yeast are used, and "acclimatization" is carried out with 1 gm.

[1] The original charge of 1 gm. of dextrose is added for the purpose of "acclimatizing" the yeast, and saturating the solution with carbon dioxide. (In case the material to be analyzed is a dilute sugar solution, this original charge of dextrose is omitted and carbon dioxide is passed through the solution until the latter is saturated.)

of maltose; otherwise the conditions for the preliminary fermentation are the same as in the determination of dextrose and laevulose. When this fermentation is complete, shaking is stopped, the gasometer is reset to zero, a weighed sample of the material to be analyzed containing not more than 4 gms. and preferably about 3 gms. of total sugar, is added to the reaction bottle which is connected to the gasometer as quickly as possible, given a few whirls by hand, and the zero reading taken. Shaking is resumed and continued until fermentation is complete, and the volume of CO_2 noted. Parallel experiments are carried out at the same time to determine the volume of CO_2 obtained per unit weight of pure standard dextrose, sucrose, and maltose with bakers' yeast.

A typical set of data and calculations are shown below:

Mycoderma Organism

2 gms. of standard dextrose gave 400 cc. of CO_2.
2 gms. of standard sucrose in presence of invertase gave 400 cc. of CO_2.
6 gms. of the sample being analyzed gave 150 cc. of CO_2 in the absence of invertase; an additional 100 cc. of CO_2 were obtained when invertase was added.
Grams of dextrose in the sample = $150 \div 400/2 = 0.75$ or 12.5%.
Grams of sucrose in the sample = $100 \div 400/2 = 0.50$ or 8.3%.

Bakers' Yeast

2 gms. of standard dextrose gave 400 cc. of CO_2 or 200 cc. per gm.
2 gms. of standard maltose gave 400 cc. of CO_2 or 200 cc. per gm.
2 gms. of standard sucrose gave 420 cc. of CO_2 or 210 cc. per gm.
10 gms. of sample being analyzed gave 600 cc. of CO_2.

Hence the cc. of gas due to dextrose equals

$$0.75 \times \frac{10}{6} \times 200 = 250,$$

and the cc. of gas due to sucrose equals

$$0.50 \times \frac{10}{6} \times 210 = 175.$$

Therefore, $600 - (250 + 175) = 175$ cc. due to other fermentable sugars designated as maltose; and $175/200 = 0.88$ gms. of maltose or 8.8%.

Using the methods of procedure as described, pure sugar mixtures of known percentage composition were analyzed. The results of these analyses are shown in Tables I and II.

TABLE I

DETERMINATION OF DEXTROSE AND SUCROSE IN PRESENCE OF A LARGE AMOUNT OF MALTOSE

Kind of sugar	Amount present	Amount found	Amount present	Amount found
	gms.	gms.	gms.	gms.
Dextrose	0.350	0.375	0.700	0.700
Sucrose	0.750	0.800	1.500	1.600
Maltose	3.900	Undetermined	7.800	Undetermined
Dextrose	0.513	0.500	0.550	0.500
Sucrose	0.500	0.475	1.500	1.400
Maltose	0.488	0.500	1.950	2.148

TABLE II

DETERMINATION OF DEXTROSE, SUCROSE, AND MALTOSE IN PRESENCE OF SMALLER AMOUNTS OF MALTOSE

Kind of sugar	Amount present	Amount found	Amount present	Amount found	Amount present	Amount found
	gms.	gms.	gms.	gms.	gms.	gms.
Dextrose	1.013	1.000	1.375	1.350	0.550	0.625
Sucrose	1.000	0.965	1.125	1.050	1.950	1.900
Maltose	0.488	0.490	0.000	0.076	0.000	0.029

A Typical Analysis by the Biological Method

A typical, condensed but complete record of the analysis of a malt syrup is next illustrated: Two solutions—one containing 25 cc. of buffer solution, 1 gm. dextrose, 5 gms. mycoderma, and 75 cc. of water; and the other, 25 cc. buffer solution, 1 gm. of maltose, 3 gms. bakers' yeast, and 75 cc. of water—are placed in separate reaction bottles in the thermostat, connected to the gasometer, zero readings taken, and shaking commenced. The production of CO_2 is shown below:

Time		Gas as read on gasometers	
		1	2
		cc.	cc.
Started	9:45	0	0
	11:15	125	140
	11:30	140	155
	11:45	140	155

At this point (11:45) shaking was stopped, 10 gms. of malt syrup was weighed into paper shells and added to No. 1, and 5 gms. of malt syrup was added to No. 2. The zero readings were taken immediately after making connection to gasometer. Shaking was then resumed.

11:45 zero reading	150	160
12:15	220	260
12:45	305	—
1:00	320	—
1:15	320	610

At this point (1:15) 2 cc. of a 5% solution of a commercial invertose preparation was added to No. 1, zero reading taken, and shaking resumed.

1:16 zero reading	340	
1:45	370	
2:00	375	
2:15	375	660
2:30	—	665
2:45		670
3:00		670
		—

Thus 320 − 150 = 170 cc. of CO_2 obtained from dextrose in 10-gm. sample,

375 − 340 = 35 cc. of CO_2 obtained from sucrose in 10-gm. sample,

and 670 − 160 = 510 cc. of CO_2 obtained from total fermentable sugars in 5-gm. sample.

Limitations of Method

It is desirable, although not absolutely necessary, to work in a constant temperature room; at any rate, the fluctuations in room temperature should be kept to a minimum. For strictly comparable work barometer variations should also be taken into consideration The thermostat was maintained at a temperature of 30 degrees ± 0.5 degrees in the investigation reported here. In mixtures of dextrose, sucrose, and maltose; dextrose and sucrose may be determined with considerable accuracy. The accuracy of the maltose determinations, however, may not be as good as in the case of dextrose and sucrose, as any errors in these determinations will be additive in their effect upon the maltose value obtained.

Precautions to be Taken in Making Readings

As the gas volumes approach constancy in any particular test, readings should be taken every 15 minutes, for after reaching a constant maximum value, the volume of gas has been found to decrease slowly. Also on account of this fact the period between the time of arriving at constant volume and the addition of invertase should not be unduly prolonged.

Conclusions

The conclusion to be drawn from a number of experiments, carried out as outlined, is that dextrose, sucrose, and total fermentable sugars in mixtures, can be estimated by this fermentation method, but that it is advisable to carry out control experiments with pure sugars before using the method for any special purposes.

The degree of accuracy should be improved, especially in the case of maltose when present in small amounts relative to the other sugars, by measuring the sugars before and after fermentation with sensitive sugar reagents; for example, by copper reduction, and optical rotation.

The method also lends itself to the detection of small amounts of invertase and maltase in plant materials. The development of a suitable active maltase preparation would increase the specificity and accuracy of the method in regard to the determination of maltose.

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STUDY OF TENDERNESS IN PASTRIES MADE FROM FLOURS OF VARYING STRENGTHS

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Introduction

Standards for excellence in pastry or pie-crust vary, but the leading considerations are tenderness, and flakiness or puffiness or lightness. Of the two, the first is possibly the more important. If one must choose between them, a tender, rich, mealy pastry is more likely to prove acceptable, at least to the sense of taste, than is a well puffed product which is hard and cracker-like and possibly tough.

This paper presents a few observations made during a study of pastry tenderness as related to type of flour used in making the pastry. The object of the study was to perfect a pastry-making test method which would yield a low coefficient of variation, with the baking of 50 wafers, from 5 mixings and bakings of the same flour, when thrown into a single series and compared. This objective, however, was only partially attained.

Experimental Work

Thirteen commercially milled flours were tested by us, viz.: Four special cake or pastry flours, most of them very finely granulated, protein content varying between 7.5% to 8%; seven general purpose or family flours from Nebraska or Minnesota mills, protein content about 10%; and two high protein flours from a California milling company, protein content 13.5% and 14.5%.

After considerable investigation the test formula as finally adopted was as follows:

Flour 170 gms.
Fat (hydrogenated) 80 gms.
Water (tap) 45 cc.
Salt (table) 3 gms.

A mixing period of 5 minutes at 60 r. p. m. (Kitchen-aid) was used to mix the fat with the flour. Water was mixed into the fat-and-flour mass for 1 minute at 60 r. p. m.

Rolling was done by hand with an 18 inch rolling pin, on a board provided with cleats ⅛ inch high. The mixing, rolling, and cutting

processes together, consumed about 15 minutes for a batch of the size described above (50 wafers).

The cutter measured 2¾ by 2¼ inches. Weight of dough in each wafer was 11.5 ± 0.2 gms.

Throughout these tests, all ingredients were at refrigerator temperature, 8° to 9° C., at start of mixing; but at the close of rolling, the dough had usually very nearly attained room temperature, which was about 22° C. during the winter months.

During the later spring months, the room temperature varied between 18° C. and 30° C. Although special efforts were made to keep dough temperature down, these were only occasionally successful.

The wafers were baked at an oven temperature of 425° F. ± 4°. The time of baking was 13 minutes. The baking pans were medium light tin 12 × 15 inches, weighing about 350 gms. Losses in weight due to baking averaged 13% ± 4%.

As the measure of tenderness, the wafers were subjected to a breaking test. For this purpose the wafers were conditioned 30 minutes on a wire rack at room temperature, then broken in a shortometer of the Bailey (Minnesota) type. In this shortometer, pressure was produced by a motor winding a cord around a spool, and was exerted against the wafer. The bars over which the wafer was broken were 13/16 inch apart.

Observations Made During Preliminary Tests with Respect to the Suitability of the Formula Used

A considerable amount of preliminary experimentation revealed that this low-fat formula discriminated better among different flours, than did a high-fat formula producing more tender wafers. A higher proportion of water in the formula would, of course, have approximated home and bake-shop conditions more nearly. Other ingredients being constant, a greater proportion of water would also have increased the breaking strength, and it might possibly have reduced the coefficient of variation. With such a change of formula, however, the dough from the finely ground low protein flours would have been too sticky to handle.

The long mixing period of 5 minutes for fat and flour gave more consistent results than did a shorter one.

As might be expected, this over-mixing produced a pastry which tended to be mealy rather than flaky, but the low coefficient of variation for breaking strength averages was considered to be of greater importance than a combination of ideal pastry qualities in the test wafer.

Slight variations in the rolling method, though at times unavoid-

able, were reflected in breaking strength tests. A suitable mechanical rolling device would be helpful in studies of this nature.

When the dough was rolled 1/8 inch thick, a greater spread among different flours, as to mean breaking-strengths, was secured, than when rolled 1 1/16 inch thick.

Conditioning or ripening of dough between manipulations or just before baking, whether at 8° C. or at 20° C., seemed to increase rather than decrease variability among duplicates and replicates.

A tendency toward inverse correlation between room and dough temperature on the one hand, and breaking strength of wafer on the other, was more or less plainly discernible as can be seen from Table I.

TABLE I

RELATION OF ROOM AND DOUGH TEMPERATURES TO THE BREAKING STRENGTHS OF EXPERIMENTALLY MADE PASTRY WAFERS

Kind of flour	Lot number	Number of wafers	Breaking strength (average)	Room temperature [1]	Dough temperature [2]	Coefficients of variation
Flour No. 1 (10.5% protein)	1	10	391	28	24	10
	2	13	389	26	23	23
	3	11	487	25	23	15
	4	13	502	25	22	15
	5	11	532	25	23	19
	6	10	574	25	22	8
	7	9	630	25	23	7
	8	10	508	24	19	15
	9	9	793	24	22	9
	10	10	840	24	23	10
	11	14	565	23	23	19
	12	12	725	22	19	18
Total	12	132	578			14 (Average)
Flour No. 2 (7.5% protein)	1	16	263	30	26	19
	2	13	397	26	22	9
	3	12	405	24	21	11
	4	14	452	24	22	11
	5	15	422	18	18	19
Total	5	70	388			14 (Average)

Note: Spring months, often no artificial heat, windows usually open.

[1] Room temperature was taken at the time the pastry was ready for rolling.
[2] Dough temperatures after rolling were lower, in many cases, than the figures given here.

It is plain that standardization of dough temperature during mixing is an important detail. Warm fat greases the flour more than does cold fat, and thus interferes with absorption of water by the gluten. This in turn causes lessening in breaking strength of the wafers. A constant temperature dough chamber for mixing and rolling would appear almost essential.

Widely varying air humidities, also, may need to be considered.

Coefficients of variation were lower when baking for 13 minutes on the medium light-weight tin tray, than when using a similar tray made of iron weighing almost twice as much, and baking for 7 minutes at the same oven temperature.

Results Obtained with Various Flours

As a means of testing the experimental method here developed, five blends were made, each blend containing two flours differing widely in strength and breaking test figures. These blends were then tested by the usual method. The experimentally determined values for breaking strength were then compared with those calculated for the blend, by averaging the two breaking strengths experimentally determined for each flour separately. As will be seen from Table II, the calculated and the experimentally determined values fall very close together, except in one case, that of the blend for flours "B" and "N."

TABLE II

AVERAGE BREAKING STRENGTHS FOR BLENDS OF STRONGER FLOURS WITH WEAKER FLOURS IN EQUAL PROPORTIONS

Flour designation	Protein percentage	Average breaking strength		
		For individual flours experimentally determined	Averages for blends	
			As calculated	Experimentally determined
A	14.5	944 [1]		
N	9.9	565		
Blend (A and N)	12.2		755+	780
M	10.1	578		
Blend (A and M)	12.3		761	788
B	13.5	936+ [1]		
Blend (B and N)	11.7		750+	612
Blend (B and M)	11.8		757	719
Y	7.96	347		
Blend (Y and M)	9.03		462	428

[1] Actual value higher than this, for a number of wafers broke under pressure too great to be measured in our machine.

Summary of Results

1. Details are given describing a method for making standard pastry wafers and testing their tenderness, or breaking strength.

2. Room and dough temperatures have a profound influence upon breaking strength tests, which decrease in numerical value as the temperature rises. Presumably this is because the warm fat greases the flour particles so thoroughly as to interfere with water absorption by gluten.

3. In spite of several obvious limitations of technique, this standardized method yielded results which proved significant from the

statistical point of view when it was employed under the comparatively uniform atmospheric conditions of artificially heated rooms carefully regulated during the winter months. In these circumstances the differences in mean breaking strengths among standard wafers made from these 13 different flours were from 3.5 to 30 times as great as their own probable errors in all cases except one.

4. The breaking strengths are rather closely correlated with protein content of flour. When the room temperature was kept at 22° ± 1.5° C., the special pastry or cake flours, protein 7.5% to 8%, showed average breaking strengths of 347 to 399; the general purpose or family flours, protein about 10%, showed mean breaking strengths of 565 to 751; and the high protein flours (about 14%) showed values above 950.

5. Five blends of high protein with equal parts of lower protein flours, when given the standard pastry wafer test, showed breaking strengths quite near the value arrived at by averaging the breaking strengths of the two flours, except in one instance. (See Table II.)

6. Coefficients of variation for sets of 50 or more wafers made from four separate mixings and bakings, ranged from 13% to 19%, when room temperature variations were not more than 1° to 3° C. These are very similar values to those calculated for a series of pastries tested by Denton and Lowe [1] with the Davis shortometer. Pastry being by nature a much looser aggregation than are most doughs, this high coefficient is perhaps not to be wondered at, though certainly it should be lowered if possible. Further study is needed.

REPORT OF THE CEREAL SECTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

J. A. LeClerc, General Referee, 1932

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For the second time in the history of the Association of Official Agricultural Chemists, which held its 48th annual meeting at the Raleigh Hotel, Washington, D. C., October 31 to November 2, the Cereal Chemists assembled as a separate section. The attendance at the meeting was about 40.

The meeting was opened by the General Referee who acted as chairman. Attention was called to the many types of investigations

[1] Belle Lowe, Experimental Cookery, p. 469. John Wiley & Sons, New York.

which are of special interest to cereal chemists, such as those associated with the production of cereals, the milling, the baking, and the manufacture of the various cereal products, and the chemical and physical changes which take place in the manufacture of these products. Mention was also made of the importance of cereals. It was pointed out that cereal crops account for nearly one-half of the value of all farm crops; that cereal foods constitute fully one-fourth of the value of all manufactured foods; that cereals supply one-fourth of our protein, and over one-third of the energy of all foods ingested by man; that both milling and baking belong to the billion dollar class of industries; and that in the utilization of the 110,000,000 barrels of flour consumed in this country, approximately $800,000,000 worth of food products, other than flour, are used, fully two-thirds of which are products of the farm. It follows that the cereal chemist occupies a position of considerable economic importance and has an unusual opportunity to be of special service to his country and his fellowman.

Interest in the reports and papers was manifest throughout the morning and afternoon sessions. A departure from the usual program was the reading of three special papers in addition to 14 reports of the Associate Referees. These papers were prepared and read by F. L. Dunlap, H. C. Gore, and Washington Platt.

The subject of Doctor Dunlap's paper was, "Tailor-made Flour," in which he brought out, that in order to process, day by day, such flour as is best suited for its purpose, the miller selects his wheat, makes blends of the various wheats available, utilizes the skill and knowledge of his chemist, controls the granulation, selects and blends the various mill streams, and often subjects his product to a certain degree of bleaching or maturing. Considerable attention was focused on the composition of flour, its food value, its ash content, the amount of P and Ca present in the ash; the quality of the proteins, fat, and its vitamin content; the effects of irradiation, etc.

A second paper, by H. C. Gore, "The Polarimetric Method for the Estimation of the Saccharogenic Power of Flour," brought out the fact that in the dough batch the following (and probably other) diastatic activities are taking place: Starch-hydration power, saccharogenic power (attack of diastase on the raw starch), liquefying power (power to liquefy cooked starch to form soluble starch), and saccharifying power (ability to transform soluble starch into reducing sugar and dextrin).

The saccharogenic power can be measured by determining the increase in Cu-reduction of a dough or flour-in-water suspension, as, for example, by the Rumsey method or by a polarimetric procedure. By the Rumsey technique the average for 10 flours was found to be 1.2 gms. of reducing substance (calculated as maltose) per 100 gms. of

flour. The Rumsey method, as modified by Gore, consisted of polarizing a flour filtrate made after the autolysis of a flour suspension. Briefly, the method described, is as follows:

To 25 gms. of flour, in a 500 cc. Erlenmeyer flask, add 341.5 cc. of Sörensen's phosphate mixture (5.288 pH at 37° C.) and incubate for 1 hour at 37° C., shaking the flask at 15-minute intervals. At the end of the incubation period, cool the flask rapidly (in ice bath) to 20° C.; add 75 cc. of Na_2WO_4 (15 gms. per 100 cc.); whirl the flask and add 1cc. H_2SO_4. Mix the contents of the flask thoroughly and polarize immediately.

The paper by Doctor Platt, "Higher Mathematics in Interpreting Baking Quality," emphasized that the term "baking quality" is really the sum of a number of entirely separate characteristics. The value of a single figure baking score was discussed in detail. It was predicted that, in the future, cereal chemists will dwell more upon protein, ash, color, and diastatic power, etc., and less upon the total baking quality, the real meaning of which is not exact. It was pointed out that many ingredients, other than flour, influence bread or cake baking qualities, and it may be desirable to determine what these are. Yeast, salt, malt, etc., all influence bread baking quality. Instead of "baking quality" cereal chemists should speak of "baking qualities."

A summary of the Associate Referees' reports follows:

1. The H-ion Concentration of Flour, *by Rowland J. Clark.* (Read by Emily Grewe.) Comparative tests of the electrometric and of the proposed colorimetric methods were made by a number of collaborators. The results indicate that the colorimetric method can be used with satisfaction. The method which follows differs from the electrometric method (Methods of Analysis, A. O. A. C., Third Edition, 1930, Chap. XX, Sec. 11) only in the last sentence. "Weigh 10 gms. of flour (or some multiple thereof) into a clean, dry Erlenmeyer flask and add for each 10 gms. of flour 100 cc. of distilled H_2O at a temperature of 25° C. Shake or whirl the flask until the particles of flour are evenly suspended and the mixture is free from lumps. Place in a thermostat at 25° C. and shake, continuously or intermittently in such a manner as to keep the flour particles in suspension, for 30 minutes. Let the flask stand quietly for 10 minutes, then decant the supernatant liquid into the colorimetric H-ion vessels and *immediately determine the hydrogen-ion concentration by comparison with suitable colorimetric standards."*

2. The Diastatic Value of Flour, *by M. J. Blish.* (Read by H. C. Gore.) Collaborative studies were made of the modified Rumsey method. The results indicate that it is unnecessary, for practical purposes, to make blank determinations, as the amount of reducing sugars originally present in sound flours is about 0.1% to 0.2%. The pro-

cedure used for the collaborative work depended upon the determination of the maltose produced after 1 hour's diastasis at 30° C. This method, however, was later supplemented by a simpler, shorter and more convenient method, the entire operation of which, including the 1-hour digestion period can be completed in 1½ hours. In this later method the sample is digested at 30° for 1 hour with a buffer solution; after which is added a small amount of H_2SO_4, and Na_2Wo_4 and the solution filtered. To 5 cc. of the filtrate exactly 10 cc. of N/20 alkaline ferricyanide solution is added and the mixture heated, in a test tube, in a boiling water bath for exactly 20 minutes. The contents of the test tube are cooled, transferred to a 250 cc. Erlenmeyer flask, 5 cc. of glacial acetic acid and 50 cc. of KI-$ZnSO_4$-NaCl solution, and 2 cc. soluble starch solution are added, and this mixture titrated with N/20 $Na_2S_2O_3$-solution.

3. STARCH IN FLOUR, *by L. Jones.* (Read by V. E. Munsey.) The modified Rask method was subjected to collaborative work. Very closely agreeing results were obtained with both patent and whole wheat flours.

4. FLOUR BLEACHING CHEMICALS, *by Dorothy Scott.* The Tentative Rask method (Methods of Analysis, A. O. A. C., Third Edition, Chap. XX, Sec. 39) somewhat modified, was further studied. The method thus modified eliminated the high blanks, the difficult end-point, and the time was shortened, thus overcoming three objections to the Seidenberg method (ibid., Chap. XX, Sec. 41). The Associate Referee also reported that a method for the detection of BzO_2 as benzoic acid in flour had been successfully worked out.

5. CO_2 IN SELF-RISING FLOUR, *by L. D. Whiting.* (Read by P. Logue.) No collaborative work was conducted. The Associate Referee reported on the various factors which may influence the results obtained by the various gasometric methods, special references being made to the published papers by Hertwig and Hicks, Chittick, Dunlap and Richards, and to his own experimental work. From the latter, the Associate Referee concludes that there is no significant differences in the amounts of CO_2 remaining in the liquid when 2 different flours are used; that the volume-reading due to vapor pressure in case of the dry flask is 3 cc., while in the case of the dry flask containing approximately 17 gms. of plain flour the reading is 5 cc. The gasometric method requires much less time than the absorption method and when the Chittick apparatus is used the former method is the preferred procedure.

6. DETERMINATION OF H_2O IN BAKED PRODUCTS, *by L. H. Bailey.* Collaborative work showed that satisfactory and comparable results are

obtained with the vacuum oven and the air oven methods (Methods of Analysis, A. O. A. C., 1930, Chap. XX, Sec. 2 and 4) in the case of bread, cake, crackers, wafers, and pretzels.

7. Lipoid P_2O_5, Fat and Water Soluble N. Precipitable by 40% Alcohol, *by L. C. Mitchell.* (Read by B. R. Jacobs.) The results of special study of the method for the determination of fat by acid hydrolysis (Methods of Analysis, A. O. A. C., 1930, Chap. XX, Sec. 8) indicate that the temperature (70° to 80° C.) is too high and of too great a range to insure concordance in the hands of different analysts. The method for the determination of P in eggs (Methods of Analysis, A. O. A. C., 1930, Chap. XXIII, Sec. 16) appears to be satisfactory for patent flour and noodles made from patent flour. Doubling the amount of Na_2CO_3 solution reduced the amount of P remaining in the charred material after leaching it with HNO_3. When the temperature is raised from 500° to 700° C. no P was found in the charred mass after leaching.

The lipoids in noodles and flour were determined by the method for lipoids in eggs, i.e., the extraction with alcohol-$CHCl_3$ was made on the wetted sample instead of the dry.

The work on the determination of the water soluble N showed that when 5 cc. of 0.01 N acetic acid per gram of noodles was used, a clear filtrate was obtained but that when 15 cc. of NaCl solution (28 gms. per 300 cc. H_2O) was added to 100 cc. of the filtrate, a copious precipitate was obtained which dissolved when made up to 200 cc. with 95% alcohol.

8. Crude Fiber, *by Ruth G. Capen.* The results obtained by 4 collaborators in the determination of fiber in bread, cake, and macaroni showed very good agreement. The method used was the official method for the determination of crude fiber in Feeds (Methods of Analysis, A. O. A. C., Chap. XXVII, Sec. 17).

9. Milk Solids in Milk Bread, *by Arnold Johnson.* (Read by B. D. Ingels.) The Associate Referee tested several methods for the determination of milk solids in bread: (1) By the loss of moisture in dry bread, the theory being that if milk has been used, dry bread when further heated to 130° will lose additional weight. This depends upon the fact that milk powder, which has previously been dried at 100°, when further heated for 3 hours at 130° loses as much as 17% of its weight. (2) By the determination of casein in bread. (3) By the determination of lactose in bread. The last named procedure was the most promising. Only the crumb was used. The sugars, other than lactose, were fermented away by treating the crumb extract with yeast.

10. Rye in Rye Flour, *by J. H. Bornmann.* (Read by M. R.

Coe.) This paper was a review of the work already published, special stress being laid upon the researches of Tillmans and of Strohecker.

11. Experimental Baking Tests, *by C. G. Harrel.* The Associate Referee approved in general the A. A. C. C. method and called attention to possible errors due to the fact (1) that the yeast used is not always of uniform strength, and that the freshness of the yeast is a critical factor; and (2) that the temperature variation during the mixing operation should be limited to $\pm 0.5°$ C.

Due to unavoidable circumstances no reports were submitted by the Associate Referees on the following subjects: (*a*) "Ash in Flour and Alimentary Paste." (*b*) "Ash and Chlorides in Baked Products." (*c*) "Color in Flour." (*d*) "Foreign Methods for Testing Flours." (*e*) "Unsaponifiable Matter in Fat of Flour and Baked Products."

The following methods were approved by the Association:

1. That the vacuum method (official first action) for the determination of H_2O in air-dried bread be made official (final action).

2. That the method for determining water in bread by heating at 130° for 1 hour (official first action) be made official (final action).

3. That both the methods cited above be made official (first action) for the determination of water in air dried products other than bread.

4. That the method for the determination of crude fiber in bread and macaroni products (official first action) be made official (final action); and that this method be made tentative for baked products other than bread.

5. That the colorimetric method, as proposed by the Associate Referee, for the determination of the hydrogen-ion concentration in flour, be made tentative.

The following recommendations were made for future work:

1. That special (non-collaborative) studies be conducted on the methods for the determination of unsaponifiable matter in the fat of flour, macaroni, and baked products, in conjunction with the same study on eggs.

2. That further study be made of the tentative method for the determination of water-soluble-protein-nitrogen precipitable by 40% alcohol in flour, macaroni, and baked products, in conjunction with similar methods for eggs. This method should be designated "Crude Albumen."

3. That comparative tests be made of foreign and domestic methods of chemical analysis used as measures of evaluating flour, macaroni, and baked products.

4. That further study be made of the method of ashing flour, macaroni, and baked products, with the object of reducing the time of combustion.

5. That the modification, proposed by the Associate Referee, for the determination of chlorine in bleached flour according to the basic Rask method be further studied; and that collaborative work be conducted on the method for the detection of benzoyl O_2 (as benzoic acid) in flour.

6. That the acid hydrolysis method, for the determination of fat in macaroni and baked products, be further studied.

7. That the method, as modified by the Associate Referee, for the determination of phosphorus in eggs, be studied collaboratively regarding its applicability to flour, macaroni, and baked products.

8. That the method, as modified by the Associate Referee, for the determination of lipoids and lipoid-P in flour, macaroni, and baked products, be further studied.

9. That further collaborative studies be made of the method, as modified by the Associate Referee, for the determination of the diastatic activity of flour.

10. That the study of methods for the determination of color in flour be resumed, special attention being paid to the use of the Munsell or similar apparatus.

11. That the Rask method for starch determination in flour modified by Munsey be further studied collaboratively; and that further work on the diastase-acid hydrolysis method be discontinued.

12. That the $CHCl_3$ test, as well as others suggested by the Associate Referee, for the detection of rye flour in wheat flour and wheat bread, be studied collaboratively.

13. That methods for the determination of CO_2 in self-rising flour be further studied.

14. That study be made of methods to determine milk solids in bread.

15. That the method for the determination of chlorides in flour and baked products (official first action) be further studied.

16. That the tentative method for making a standard baking test be further studied.

17. That the tentative method for the preparation of samples of macaroni products for analysis be further studied.

18. That further collaborative work be conducted with the method of determining crude fiber in baked products other than bread.

19. That further studies be made to determine what color standard is best adapted for flour pH work.

20. That the applicability of the color method to the hydrogen-ion concentration of macaroni and baked products be studied.

Annual Report of Secretary-Treasurer

M. D. Mize

January 1, 1933

The same general form that has been used during the past two years has been retained in compiling this year's report. Therefore, a very good comparison of Association activities this year can be obtained by referring to the last two previous reports printed in Cereal Chemistry. Your Secretary-Treasurer sincerely hopes that the entire report is self explanatory and that there is no necessity for greater detail in explaining any item in the report.

We might take the liberty of calling your attention to the fact that this is the first time during the past five or six years that the Association has not experienced a net gain in membership. The net loss of four members figures less than 1% and when one stops to think that many organizations have sustained a loss from 25% to 50% in their activities during 1932, it must be conceded that the Association has more than held its own.

DETAILED MEMBERSHIP STATEMENT DECEMBER 31, 1932

	Total	Active	Corp.	Hon.
Membership, Dec. 31, 1931	464	414	48	2
New members added during 1932	41	38	3	–
Members reinstated	3	3	0	0
	508	455	51	2
Members resigned and suspended for non-payment of dues during 1932	47	42	5	–
Members deceased	1	1	–	–
Members in good standing Dec. 31, 1932	460	412	46	2
	508	455	51	2

PROFIT AND LOSS STATEMENT

January 1 to December 31, 1932

RECEIPTS 1932			
Cereal Chemistry			
Membership dues			
Active		$1,442.00	
Corpporation		460.00	
Subscriptions, reprints, backnumbers and advertising	$4,051.12		
1932 Subscriptions received 1931	413.25		
1932 Accounts receivable	89.22		
1933 Income received in 1932	*729.21*		
1931 Income received in 1932	*189.13*		
Net 1932		3,635.25	
Interest on Invested Funds		70.66	
Total Net Receipts 1932			$5,607.91

Association			
Membership dues		1,441.56	
Application Fees		114.00	
Interest on Invested Funds		58.49	
Miscellaneous Income		10.00	
Total Net Receipts 1932			1,624.05
Experimental Baking Fellowing Fund Refund From U. of N. and interest			62.98
TOTAL RECEIPTS OF ALL ACCOUNTS 1932			$7,294.94
DISBURSEMENTS 1932			
Cereal Chemistry			
Cost of editing, printing and mailing Cereal Chemistry and reprints	$5,909.64		
1932 Accounts Payable	123.14		
1931 Accounts paid in 1932	*651.71*		
Net Disbursements 1932		$5,381.07	
Profit 1932			$ 226.84
Association			
Expenses of President, Vice-President Office, News Letters	189.59		
Expenses of Sec'y-Treas.'s Office	375.93		
Committee Expenses	36.35		
Detroit Convention Expenses	193.98		
Detroit Convention Report	301.00		
Miscellaneous Expenses	97.76		
1932 Accounts Payable	150.00		
Net Disbursements 1932		1,344.61	
Profit 1932			279.44
Experimental Baking Fellowship Fund		424.00	
Net Disbursements 1932			*361.02*
TOTAL DISBURSEMENTS OF ALL ACCOUNTS		$7,149.68	

DISTRIBUTION OF NET ASSETS

Cereal Chemistry Assets 1931	$2,770.59	
Profit 1932	*226.84*	
Assets Dec. 31, 1932		$2,997.43
Association Assets 1931	1,865.81	
Profit 1932	279.44	
Assets Dec. 31, 1932		2,145.25
Book of Methods Reserve Fund 1931	375.98	
Assets Dec. 31, 1932		375.98
Convention Reserve Fund 1931	452.78	
Assets Dec. 31, 1932		452.78
Experimental Laboratory Baking Fund 1931	444.81	
Net Disbursements 1932	*361.02*	
Assets Dec. 31, 1932		83.79
Total Assets Dec. 31, 1932		$6,055.23

FINANCIAL STATEMENT DECEMBER 31, 1932

U. S. National Bank—Checking Account	$ 104.99
Cash on Hand	992.43
U. S. National Bank—Savings Dept.	261.71
First National Bank—Savings Dept.	909.23
Petty Cash Fund in Washington, D. C.	200.00
Building & Loan Stock in Kansas City	2,000.00
Building & Loan Stock in Omaha	1,000.00
Building & Loan Stock in Minneapolis	1,500.00
1932 Accounts Receivable	89.22
GROSS ASSETS	7,057.58
LIABILITIES	
1932 Accounts Payable	*273.14*
1933 Income Received in 1932	*729.21*
NET ASSETS	$6,055.23

Note: All amounts in italics are negative amounts and are subtracted from the other amounts in the same column.

REPORT OF AUDITING COMMITTEE

W. L. Heald, *Chairman*

The Auditing Committee has examined the books of the Secretary-Treasurer for the fiscal year January 1, 1932 to December 31, 1932 and find the same to be correct.

As was recommended in 1932, this Auditing Committee has investigated the fixed assets of the Association and find all to be in sound and liquid condition with the exception of one which has been in the process of liquidation since April, 1932.

BOOK REVIEW

Agricultural Russia and the Wheat Problem. By Vladimir P. Timoshenko. Published by Food Research Institute, Stanford University, California, 1932. xi + 571 pp., 4 maps, 15 x 23 cm. Price $4.00.

Doctor Timoshenko's book is of particular interest now when the granaries of the world are bulging with the bread grains. World supplies are still abnormally large and there is no immediate prospect of reduction. As Russia has popularly been considered a source of additional surpluses, this survey of its potential productive capacity is timely.

To properly analyze the grain situation, Doctor Timoshenko has developed a thorough and complete background of Russian agriculture. To this end the physical, social, political, and economic conditions surrounding agriculture in Russia are discussed in detail. The historical background presented in this discussion is particularly valuable.

In treating of the physical conditions influencing grain production a comprehensive description of the soil, orography, temperature, and rainfall is given. Methods of seed-bed preparation, crop rotations, harvesting, thrashing, and types of machinery used by the peasants and on the state farms are detailed. The evolution and organization of collective and state farms are ably discussed.

The social conditions of agricultural Russia are presented by giving a history of the peasant class along with data describing population trends, density of population, type of settlement, etc.

The discussion of the Agrarian Revolution and the Soviet agricultural policy gives a good picture of the unstable conditions under which Russian agriculture must develop.

Land utilization, farm labor, marketing practices, transportation, storage, and milling of wheat form the subject matter of the economic discussions.

In connection with milling, several pages are also devoted to a general description of the milling and baking properties of Russian wheats. Data on domestic consumption and shipments of flour, post-war collections, and the exportation of grain and flour complete the study.

The author states that although the present moment is unpropitious for drawing definite conclusions as to further development of agricultural production in the U. S. S. R. on account of the fact that Russian agricultural organization is now in a period of transition from small scale farming to large scale farming on cooperative or socialistic lines, there is, however, some possibility of formulating the outlook for further development of agricultural production and exports, with reference to the bread grains, if we use as a basis the natural and geographic conditions of the U. S. S. R., the long time population growth, and the historical tendency toward diversification of farming.

The growth of population in the U. S. S. R. has been very rapid in recent years, 2% yearly or a little higher. With this high rate of growth of population not only would it be necessary to increase grain production by 20% in order to reach the pre-war per capita level, but it would also be necessary to further increase grain production 2% yearly to maintain it. There appears to be little possibility of increasing production to this level by increasing the crop area in bread grains. Most of the arable land of Russia was in cultivation before the war and because of the limitations of rainfall and temperature there is a very small amount of new, unoccupied land available. The bread grains now occupy over 70% of the total grain area so it is improbable that the bread grains will increase at the expense of the other crops, in fact with increased diversification of farming and improved crop rotations the percentage of the crop area in bread grains will tend to decrease. Deficiency of draft power is another factor that creates serious difficulties in immediate expansion of agricultural production. Lack of adequate transportation facilities is also a handicap to expansion, particularly to the new lands. Through seed selection and improved tillage methods, the author believes an increase in yield of about 2% per year is possible. This increases in production, the author points out, would only take care of the increase in domestic consumption resulting from the yearly increase in population.

In summing up the points as to Russia's agricultural possibilities, the reviewer presumes that it is highly improbable that Russia will regain her pre-war position in the world wheat market.

The book contains a very complete set of appendix tables giving data on land utilization, population, areas and yields of grains by regions; exports by crop years and by destinations, and grain collections by the U. S. S. R.

D. J. Martin.